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TABLE OF CONTENTS

Page

Prokof'yeva-Bel'govskaya, A. A. Interaction of Nucleus and Cytoplasm in Amylogenic Cells of Potato Tubers 1

Zhirmunskiy, A. V. Heat Resistance of Actinia and Their Ciliated Epithelium under Natural Conditions and after Experimental Changes in Environmental Temperature 21

Yermilova, V. V. Fixing of Phenol Red by Live and Killed Animal Tissues under Conditions of Diffusion Equilibrium . . 32

Ushakov, V. B. Accommodation Power of Tonic and Tetanic Muscles and Single Muscle Fibers from Frogs 38

Zander, N. V. Effect of Alkali Metal Chlorides on the Quiescent Current from the Skeletal Muscle of Frogs 47

Il'ina, L. I. and Petrov, R. V. Characteristics of Protein Synthesis in the Organoids Cells in the Tissues of Normal and Irradiated White Rats 55

Page

Olenov, Yu. M., Galkovskaya, K. F., and Pushnitsyna, A. D. Materials Describing the Effect of Ionizing Radiation on Individual Development 60

Korogodin, V. I., Malinovskiy, O. V., Poryadkova, N. A., and Izmozherov, N. A. Reversibility of Different Forms of Radiation Injury in Diploid Yeast Cells. 79

Izmozherov, N. A. Effect of Gamma-Rays on Mitosis in a Polyploidic Series of Wheat 95

Khvostova, V. V., and Delone, N. L. Radiosensitivity of the Meristem of Buds and Radicles in the Germs of Peas and Barley 103

Yushkevich, N. L. Distribution of the Protein Tryptophane in the Tissues of Large Embryos and in Certain Organs of Adult Birds 108

Astaurov, B. L., Golysheva, M. D., and Roginskaya, I. S. Chromosome Complex of the Ussuri Geographical Race of Wild Mulberry Silkworm Moths Bombyx Mandarina M. in Connection with the Origin of the Domesticated Mulberry Silkworm Moth Bombyx Mori L. 114

Sukhanova, K. M. A Cytological Characterization of Protoopalina Caudata Zeller 122

Page

BOOK REVIEW

Troshina, V. P. G. Yu. Belitskiy Ionnye mekhanizmy osnovnykh nervnykh protsessov [Ionic Mechanisms of the Basic Nervous Processes] 132

NEWS ITEMS

Vorob'yev, V. I. International Symposium (Brussels) on the Relationships between Nucleus and Cytoplasm. 139

Suzdal'skaya, I. P. and Troshina, V. P. Conference on Adaptational Reactions and Methods of Increasing Resistance of the Organisms to Unfavorable Influences. 144

For the Information of Writers 151

Correction 153

Academy of Sciences of the USSR Department of Biological Sciences 154

THE INTERACTION OF NUCLEUS AND CYTOPLASM IN AMYLOGENIC CELLS OF POTATO TUBERS

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The interaction of nucleus and cytoplasm is a central problem in modern biology.

Numerous research workers have established that the nucleus plays an important role in the synthesis of ribonucleic acid, which is essential both for building the structures necessary for life--microsomes, mitochrondia, and plastids--and for their functioning (protein, synthesis fermentation). As, being obviously the place where conferments are formed, the nucleus also participates in oxidation and phosphorylation processes.

A large number of studies have been devoted to the interaction of nucleus and cytoplasm in the processes of cell protein synthesis. (Caspersson, 1947, 1950; Hyden, 1947; Kedrovskiy, 1951 a, b, 1958; Konarev, 1954; Brashe, 1955, and others)

The peculiarities of the structure and properties of the nucleus in connection with the formation of such substances as starch, glycogen, and fats have been studied much less.

In 1901, Gerasimov in his celebrated experiments with Spirogyra algae was the first to discover that cells without nuclei have the ability to synthesize starch. Studies of the functioning of liver cells have shown that during mitosis their glycogen and fatty inclusions, with which the neighboring cells in a state of "rest" were filled disappear (Ortiz-Picon, 1933). The intensive formation of starch by the cells of the endosperm of cereals results in destruction of the nucleus (Aleksandrov and Aleksandrova, 1938, 1939, 1954).

The starch-forming cells of potato tubers are of great interest in studying the interaction of nucleus and cytoplasm in starch-forming processes. Research in recent years has established a close connection between the starch-forming activities of the amyloplastids in the cells of the storage parenchyma of potato tubers and the state of the cellular nucleus (Aleksandrov, Yaklovlev, and Klimochkina, 1947; Prokof'yeva-Bel'govskaya, 1953; Aleksandrov and Aleksandrova, 1954).

This work is a comparative cytological study of the amylogenic cells of healthy and degenerate potato tubers. The behaviorial pecularities of the nuclear and cytoplasmic structures revealed here are of interest both in respect to the general problem of interaction of nucleus and cytoplasm and in respect to the nature of potato degeneration, which has until recently been a subject of discussion (Linnik, 1955).

Materials and Methods

Healthy and degenerate potato tubers of the Early Rose variety in various periods of their development planted by the Institute of Genetics, Academy of Sciences USSR in the Kirgiz SSR (1942 and 1943) and in Moscow (1944 and 1945) served as material for the studies. Small sections of the hearts of the tubers, cortical parenchyma, and eyes were fixed in 96° alcohol (Carnoy's fixing fluid), chromacetoformol (Navashin), and mercuric chloride with formalin (Helly's fixing fluid). An analysis of the morphological features was made on preparations stained with Heidenhain's [iron] hematoxylin and gentian violet.

To show up the ribonucleic acid (RNA) sections (10-15 microns) were stained with pyronin (along with Unna's methyl green); desoxyribonucleic acid (DNA) was revealed with the aid of a nuclear reaction and staining with methyl green and pyronin.

The Meristem of the Eyes

Healthy Early Rose Tubers

The eyes of a mature potato tuber consist of a well-developed center point of growth and two less developed, lateral points. The cells of the meristem are in a dormant state. The cytoplasm is slightly basophile and its capacity to be stained with pyronin is greatly decreased. The nucleus contains one or two small nucleoles which are sharply colored with hematoxylin, but weakly stained by pyronin. The contained nuclei are structuralized: the chromatin forms several extremely tiny lumps which yield a weakly nucleal reaction. In some nuclei the chromatin is scarcely revealed and they appear to be "empty". The Paryenchyma is not stained with basic stains (Figure 1).

Thus, when the mature tuber enters a period of rest for the cells of the meristem of the points of growth of the eyes, like, as is obvious, the cells of any dormant meristem, contain very little ribonucleic acid in the cytoplasm and in the nucleolus or desoxynucleic acid in the chromatin elements of the nucleus. In this respect our data are compatible with Satarova's data (1950).

Degenerate Tubers

The cytological structure of the meristem of the points of growth of the eyes in degenerate tubers differs sharply from the structure described above of the eyes of healthy tubers.

The cytoplasm in many cells is strongly basophile. The nucleus is roughly structuralized like that observed in the prophase of division. The structural nature of the nucleus is caused by the presence of a large number of basophile chromatin lumps which yield a clear-cut

nuclear reaction, whose number (from 11 to 30) and size vary exceedingly in the nuclei of different cells (Figure 2). Cells with roughly structuralized nuclei and basophile cytoplasm frequently form large nidi among normal cells of the meristem of an eye.

Such a structure of the meristem provides evidence of the transition of its cells from a dormant state to a state of clearly marked prophase of division.

In some eyes of the degenerate tubers the change from the normal state of the cells was limited to this: the cells were not dividing and their nuclei presented a picture, so to speak, of a fixed, prolonged prophase, that is, the cells entered the first phase of division and stopped at that development of the process (Figure 2a). However, many of the eyes under study behaved otherwise: the DNA content in the nucleus (according to nuclear reaction) was increasing sharply, the process continued further, and the cells entered a state of active mitosis, out of which they could not go for a protracted period of time. Numerous pictures of different phases of mitotic division were seen in sections of such eyes. The dormant stage of the cells was shortened exceedingly, and typical dormant nuclei were almost never observed (Figure 2,b). Mitoses were proceeding throughout all sections of the meristem of the eye, occurring in the soft part of the eye, then later in the parenchyma of the tuber. Sections of tissue with such cells are foci for growth of the tuber.

The cells of eyes in degenerate tubers possess still another exceedingly clear-cut peculiarity in some meristematic foci--an extremely high diffuse basophilia of the basic contained nucleus and cytoplasm. Chromatin lumps in the nuclei with diffuse basophilia of the karyenchyma are weakly colored and have the appearance of friable bodies with unclear markings.

The following change applies to the nucleolus. In many nuclei it is enlarged, which points to its premitotic activization (Navashin and Makushenko, 1956); it is clearly stained with hematoxylin and pyronin. In cells with diffuse basophilia it is stained more weakly. Groups of cells with clearly colored nucleoli which had attained gigantic size (Figure 3) were found in the meristem and the parenchyma of eyes from markedly degenerate tubers. The basic content of the nucleus with a similar nucleolus was forced toward the membrane of the nucleus by a wide band. The chromatin lumps were small and few in number. Groups of cells with gigantic nucleoli were found in sections of the meristem along with cells which contained structuralized nuclei and cells which had entered a state of mitosis.

It is necessary to note that individual eyes in a single degenerate tuber differed from each other to an exceedingly great extent in respect to the degree in which all the above described changes in the state of the meristem cells were shown. Even in tubers with clear-cut indications of degeneracy, a majority of whose eyes had meristem formed by cells which were in a state of mitosis or with gigantic nucleoli, one

found individual eyes whose meristem was formed with normal cells in a dormant state.

The material cited indicates that the cytoplasm and the nucleole of nondividing cells of the meristem of the points of growth of eyes in degenerate tubers contain a high concentration of RNA; and the chromatin elements are rich in DNA. The majority of the cells are in a premitotic or mitotic state, which also determines the "growth" of degenerate tubers.

Starch-Forming Cells
Tubers of a Healthy Sort

The replacement of mitosis by amitosis in early stages of development of tubers. The appearance of meristematic cells of a stolon during differentiation and development of a tuber is linked with intensive growth of cells and the appearance in the cytoplasm of numerous leucoplasts which form grains of starch.

These processes caused by a radical change in the character of the exchange in the cell are accompanied by a celebrated and regular change in the properties of the nucleus and cytoplasm and the cycle of development of the entire cell: the mitotic division which is usual for meristematic cells is replaced by amitosis. Cytological material is set forth according to peculiarities of amitosis in the starch-forming cells of potato tubers, its mechanism and biological significance, in an earlier work (Prokof'yeva-Bel'govskaya, 1953). Two peculiarities of amitotically dividing cells are of importance in the problem under study here of starch formation: 1) the amitotically dividing nucleus is in a state of deep dormancy--its DNA content (according to nuclear reaction) is sharply reduced; 2) intensive development of starch grains is going on in amitotically dividing cells as well as in the cells which are in a dormant state.

These facts have provided grounds for concluding that the fundamental significance of amitosis, which assures its wide propagation in differentiated vegetable and animal tissues, consists of the fact that, in contrast to mitosis (Roskin and Struve, 1948; Zalkind, 1952), without destroying the exchange of substances and without taking the cell out of its "working" state, amitotic division ensures the reproduction of cells and the growth of an organ during a period of intensive functioning (Prokof'yeva-Bel'govskaya, 1953).

Thus the cell starts to form starch under conditions of the full appearance of mitosis and, consequently, starch formation is linked in some manner with products of the activity of the "dormant" nucleus. This assumption, as we shall see later, is confirmed by cytological analysis of degenerate potato tubers.

Changes in the Nucleus in the Process of Growth and Functioning of Amylogenic Cells. Throughout the entire period of growth of the potato, the cellular nuclei do not leave the "working" state ("dormant"

state). With the growth of the tuber, however, during the process of intensive starch formation, they undergo clear-cut, regular changes (Prokof'yeva-Bel'govskaya, 1945, 1953).

In the cells of the skin parenchyma of potatoes 2-8 millimeters in diameter, the nucleus has a proper spherical form and is about eight times as large, in respect to volume, as the nuclei of meristematic cells. In the nucleus are to be seen small chromatin lumps and a delicately colored reticule which are typical of the "dormant" nucleus. The nucleolus is small, lies directly in the nucleoplasm, is intensely stained by pyronin; the karyenchyma is scarcely stained, and is transparent. The chromatin lumps yield a clear-cut nuclear reaction.

During this period the numerous starch-forming plastids which are scattered throughout the entire cytoplasm have a definite tendency to group about the nucleus (Figure 4)--a phenomenon which is well known in many vegetable cells. Observations of the dynamics of the development of starch grains show that this process proceeds most intensively in the zone of cytoplasm directly adjacent to the nucleus. Here the starch grains have already attained large size at the time that only the first layers of starch have begun to appear in the plastics of the peripheral parts of the cell. Apparently the functioning of the starch-forming plastids are linked in a very close manner with the products of the activities of the "dormant" nucleus which are concentrated near its surface. As the cells are filled with growing starch grains and are increasing in size, signs of qualitative changes begin to appear in the nuclei which, increasing progressively, lead to senescence of the nucleus. The delicate chromatin structures of the nucleus are gradually transformed into coarser, weakly basophile threads and lumps; the nucleoplasm decreases in volume, the nucleus loses its properly spherical form and becomes a somewhat angular, extended, and strongly paddle-shaped body. The nucleolus increases in size, its pyroninophilia increases and remains at a high level for a prolonged period of time.

By staining sections of the skin parenchyma which are in a period of intensive starch formation with methyl green - pyronin, one discovers a remarkable connection between the nucleus and the cytoplasm and the developing starch grains (Figure 5). The nucleus is flattened out over the surface of large starch grains. The nucleolus has a sharp purple color, which indicates a high RNA content. The nucleoplasm, which is bluish in the central part of the nucleus, acquires a rosy tint toward the periphery, increasing sharply in intensity close to the nuclear membrane. The peripheral zones of the nucleus, which form tongue-shaped projections, are sharply basophile, stained by pyronin to a clear purple color and merge with a thin layer of the basophile cytoplasm which envelopes the starch grains. The thin membrane of the stretched body of the plastid possesses the same high degree of pyroninophilia at the surface of the starch grains as the nucleolus and the peripheral zones of the nucleus.

Thus, during the period of intensive starch formation, the nucleolus, the peripheral zones of the paddle-shaped nucleus, and the thin body membrances of the starch-forming plastids contain a high concentration of RNA. It is also possible that during this period the peripheral zones of the nucleus are enriched by depolymerized DNA, which, according to Kurnick's data (Kurnick, 1953), are stained by methyl green-pyronin to a rosy or purple color, analogous to RNA. The peculiarities of the nuclear structure in the prolongedly functioning starch-forming cells of the skin parenchyma are clear-cut in spring tubers when they are sprouting. The nucleus has an irregular form and is clearly polarized: at one pole of the nucleus, where the membrane is sufficiently clear-cut, there is a greatly enlarged nucleolus which is surrounded by a wide band, its pyroninophilia is greatly reduced. At the opposite pole the nuclear membrane is much thinned out and the contained nucleus, which forms tongue-shaped protrusions, almost merges with the cytoplasm; the chromatin has the appearance of the lumps or threads of the prophase, and is weakly stained by a basic stain. At times the nucleus contains, in addition to the basic large nucleolus, numerous small nucleoli which are apparently discharged into the cytoplasm (Figure 6).

The structure and the properties of the nuclei of the cells of the storage parenchyma of potato tubers during the period of starch formation and at its completion are very like those observed in the nuclei of the intensively functioning cells of other tissues--the cells of fatty tissue of Ditiscus, cells of the spinal ganglia of Lophius piscatorius (Hyden, 1947). Such a nuclear structure permits one to conclude that during the process of starch formation there is an intense interaction of the substances in the nucleus and the cytoplasm, as a result of which the peripheral zone of the nucleus, and the parts of the cytoplasm and the body of plastids lying close to it are enriched with RNA. By the end of the starch-forming process the RNA content drops sharply in all these structures.

When the tuber germinates, the nuclei in the cells of the cortical parenchyma are resorbed, enriching the cytoplasm with the products of its decomposition. In the interior of the tuber, according to data from Aleksandrov and his co-workers (1947), and our own observations, the nucleus is destroyed considerably earlier, during the period of intensive starch formation and the final phases of this process continue in a cell with a nucleus, apparently with the participation of the products of its previous activity which are available in the cytoplasm.

According to data from Aleksandrov and his co-workers (1947, 1954), destruction of the nucleus entails an irreversible degeneration of the plastids and stimulation of the formation in the cytoplasm, with the participation of the chrondriosomes, small grains of "chrondriosome" starch. The latter conclusion is probably mistaken--apparently the fine-grained starch is formed by young plastids which have developed from proplastids (Strugger and Perner, 1956) after the nucleus has been destroyed.

Degenerate Tubers

The cells of the skin parenchyma of degenerate tubers have a number of characteristic peculiarities which sharply distinguish them from the starch-forming cells of healthy tubers.

Suppression of the replacement of mitosis by amitosis. The first peculiarity is revealed by studying young tubers. In the cortical parenchyma of healthy tubers, the cells enter the phase of intensive growth and starch formation with an orderly transition to the amitotic cycle. In the cortical parenchyma of degenerate tubers one can observe a marked destruction of this orderliness--in young tubers of 4-8 millimeters in diameter and even in larger tubers, one can observe in the cells of the skin parenchyma numerous nuclei which are in a mitotic state (Figure 7, a - c).

At times, even at this stage, mitotic division of the nucleus is accompanied by simultaneous division of the cell, but binucleate division occurs more frequently. The nuclei in binuclear cells may, in turn, undergo nitotic division. Multinuclear cells occur in the cortical parenchyma as a result of successive mitoses (Figure 7d). Mitoses of nuclei with doubled sets and paired chromosomes were found in the starch-forming cells of some degenerate tubers (Figure 7c). Consequently, having undergone one cycle of reproduction of its components without mitosis (endomitotically), the nucleus again made the transition into a mitotic state unusual for the cortical parenchyma.

Sharp differences in the cycles of development of different cells is very characteristic in the cortical parenchyma of degenerate tubers--within the bounds of a single section one can observe cells with two mitotic nuclei, restitution mitoses, as a result of which anaphase and telophase groups of chromosomes are united in a single complex figure (Figure 7b), endomitotic nuclei with the paired arrangement of chromosome elements characteristic of endomitosis, and, finally, at times one encounters cells of the parenchyma of healthy tubers. These differences in cells also determine the deformed shape of degenerate tubers.

Starch formation in all the observed cells is closely connected with the peculiarities of the cycle of the nucleus--single cells with "dormant" or amitotically dividing nuclei contain starch grains. Cells of the cortical parenchyma with mitotic nuclei do not "work," they contain large vacuoles; in the cytoplasm which forms the layer adjacent to the wall and ties between the vacuoles lie many leucoplasts which do not form starch grains; starch formation is sharply depressed (Figure 7a).

Premature "senescense" of the nucleus in starch-forming cells of young tubers. In the cortical parenchyma of degenerate young tubers (4-8 millimeters in diameter) one may observe a very noteworthy picture--in cells which contain leucoplasts and only single weakly developed starch grains one encounters, along with mitotic nuclei, numerous nuclei

with clear indications of premature "senescence (Figure 8 a - c). The nucleus is not turgescent, of irregular form, its contents are stained in a diffuse manner with basic stain, the chromatin has the appearance of weakly colored lumps, the nucleolus is enlarged, surrounded by a band, and its pyroninophilia is reduced. Starch formation in young cells with such "aged" nuclei is sharply suppressed. Numerous plastids are grouped about the nucleus; however, one can observe a weak formation of starch in single units. Apparently the suppression of starch-forming activities of the amyloplasts is connected with the low activity of the prematurely senescent nucleus, depression of the formation of RNA in the nucleolus, and the small amount (at the surface of the nucleus) of the products of its vital activity which are essential for normal functioning of the amyloplasts.

In the cells of the cortical parenchyma of healthy tubers, the nucleus acquires these indications of senescence only after a protracted period of intensive starch formation and "senescent" nuclei are discovered for the first time in tubers whose cells are filled with large starch grains.

Starch formation is most sharply depressed in cells with small, extended nuclei with basophile nucleoplasm and a small nucleolus. As a rule, such nuclei are located directly by the cell wall--around them are either no developing starch grains at all or the latter are single units and weakly developed (Figure 8 d - f).

The cells of the cortical parenchyma of mature degenerate tubers contain few, weakly-developed starch grains, among which an "aged" nucleus is located at random in a weakly colored cytoplasm (Figure 9). It is wrinkled, its basophile envelope lies in folds about its surface, it has a large nucleolus which is weakly stained with pyronin, the chromatin has the appearance of weakly basophile bodies or threads, and leucoplasts which do not form starch grains lie adjacent to the nucleus. Many cells which have attained a large size contain only single starch grains, therefore seem empty. Even in this period one can observe in some tubers cells with nuclei which are in a mitotic state.

Discussion

Cytological and cytochemical studies of healthy and degenerate potato tubers established a clear-cut dependence of starch-forming activities upon the products of the activity of the dormant nucleus.

Summing up the material available in literature (Heitz, 1925; Aleksandrov and his co-workers, 1947, 1954; Konarev, 1958), generally known facts, and data from this research, we have at our disposal the following facts which indicate the significance of the nucleus in the development and functioning of the starch-forming plastids.

1. Localization of the main mass of the plastids and their most rapid development in the zone of cytoplasm immediately adjacent to the nucleus.

2. The plastids which are located in closest proximity to the nucleus, embraced by its paddle-shaped protrusions, are the most rapidly filled with starch.

3. The intensive formation of starch by the amyloplasts and the growth of the starch grains are connected with a sharp increase in the size of the nucleolus and high RNA content (revealed by staining with pyronin) in the nucleolus, the peripheral parts of the nucleus, the cytoplasm adjacent to it, and in the thin membrane of the body of the plastid, stretched over the surface of the starch grain. By the end of the starch-forming process, the RNA content of all these structures is greatly reduced.

4. Transition of the nucleus to a mitotic state, linked with its DNA content, the discontinuance of the functioning and the disappearance of the nucleolus, are accompanied with a sharp decline in the starch-forming activities of the plastids or even their destruction (Heitz, 1925; Navashin and Makushenko, 1956).

5. Premature senescence of the nucleus, accompanied by a sharp decline in the RNA content of the nucleolus linked with a strong suppression of the development of the amyloplasts grouped about the nucleus.

6. Fine-grained starch is developed when the cytoplasm is enriched by the products of the resorption of the nucleus.

7. If the nucleus undergoes an oxalate degeneration which blocks the chromatin and nucleic acids, fine-grained starch does not develop when the nucleus is destroyed (Aleksandrov, Yakovlev, and Klimochkina, 1947).

These facts permit one to conclude that the nucleus plays a vital role in the process of starch formation. Analyzing the possible ways in which it may participate in this process, we proceed from the modern ideas as to how the functioning of the ferment systems of the plastids as well as the development and the reproduction of these structures is connected with their RNA content. The presence of RNA in the leucoplasts of sugar beet roots and leaves and its importance in the synthesis of the catalytic proteins of the plastids was shown recently in a number of works by Sisakyan and his co-workers (1952, 1954). Although the place of the synthesis of RNA in the cell has not been finally established--whether it exists independently in the nucleus and cytoplasm or in the cytoplasm with contact interaction between it and the nucleus (Belozerskiy, 1944; Caspersson, 1947, 1950; Brashe, 1955), the vital role of the nucleus in the synthesis of RNA is not doubted at present. Thus, the importance of the nucleus in the starch-forming activities of the plastids is determined by its importance in the synthesis of RNA, which is essential in the development of leucoplasts and the synthesis of the catalytic proteins contained in them.

The previously-described facts of the formation of starch by cells without nuclei (Gerasimov, 1901) apparently can be explained by a prolonged support of the activities of the plastids by the products of the previous activity of the nucleus, like that observed in Acetabularia.

Cytological study of the amylogenic cells of degenerate potato tubers shows that their nuclei undergo premature "senescence"--in young cells with plastids which have scarcely started starch formation, the nuclei already have all the signs, according to morphological and cytochemical indices, of advanced "senescence."

Degenerate potato tubers are also distinctly marked by suppression of the orderly transition of the cellular cycle from mitosis to amitosis, which is inherent in the starch-forming cells of healthy tubers.

In the cortical parenchyma of degenerate tubers we have a demonstrated example of the fact that mitotic division of the cell, which is connected with an increased DNA content in the nucleus, the development of chromosome bodies, and a general alteration in exchange, leads to suppression of the fermentative processes which cause starch formation. These fermentative processes come into action when the cell is in a state of dormancy [resting?] or amitotic division.

Being the most important agent of mitotic transformations in the nucleus and the cytoplasm, RNA also participates directly in the synthesis of the structural and catalytic proteins of the cell (Kedrovskiy, 1951 a; Roskin and Kirpichnikova, 1952; Brashe, 1955).

This complex role of the nucleotides and nucleic acids is also, apparently, one of the causes of the antagonism between mitosis and starch formation, which is clearly demonstrated in the starch-forming cells of potato tubers.

Conclusions

1. Cytological and cytochemical study of the amylogenic cells of healthy and degenerate potato tubers shows that the starch-forming activities of the amyloplasts are closely connected with the products of the activity of the nucleus, which is in a state of dormancy or amitosis in these cells.

2. There are grounds for assuming that the significance of the nucleus in starch formation is determined by its significance in the synthesis of RNA, which is essential to the development of plastids and the synthesis of the catalytic proteins which are contained in them.

* * *

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FIGURE APPENDIX

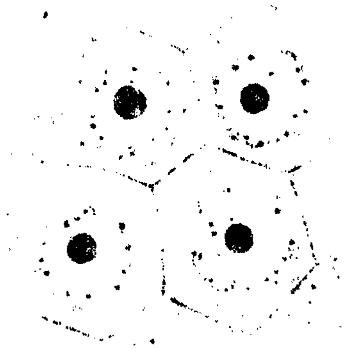
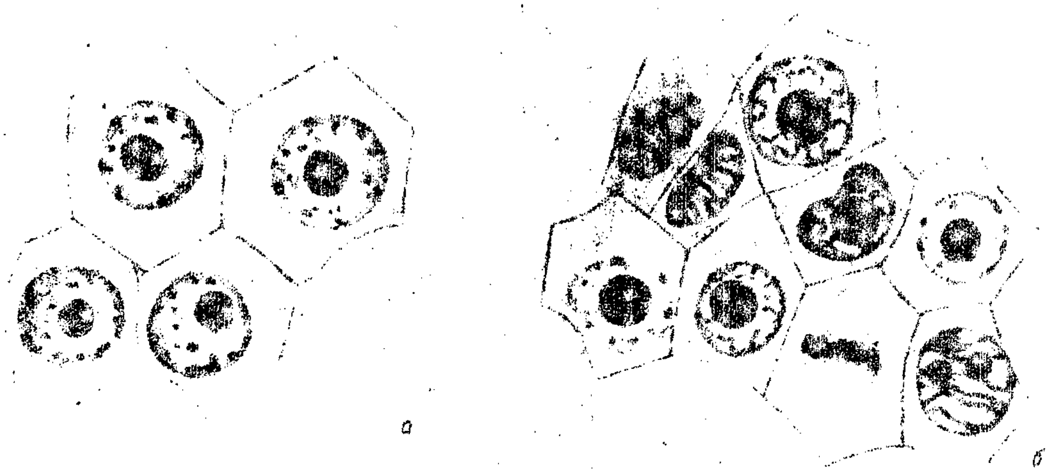


Figure 1. Section of the meristem of the eye of a healthy potato tuber. The cells are in a dormant state, the nucleus contains very fine lumps of chromatin, the nuclear reaction is negative, pyroninophilia of the cytoplasm and nucleolus is weak (X 2240).



a b

Figure 2. Section of the meristem of the eye of a degenerate potato tuber.
a - cells in prophase of mitosis. The nucleus is roughly structuralized. Large lumps of chromatin show a clear nuclear reaction. Pyroninophilia of the cytoplasm and nucleolus are decidedly clear.
b - cells are in the process of dividing. (x2100).

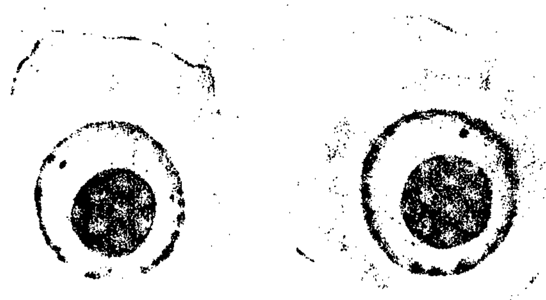


Figure 3. Cells of the parenchyma of the eye of a degenerate potato tuber. The nucleus contains greatly enlarged nucleoli (X 2240).

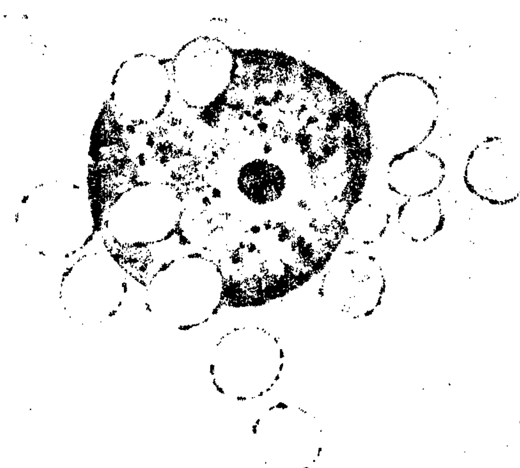


Figure 4. Cells of the cortical parenchyma of a young, healthy tuber. The nucleus is spherical, surrounded by growing plastids. The bodies of the plastids are stained brightly with pyronin (X 2240).

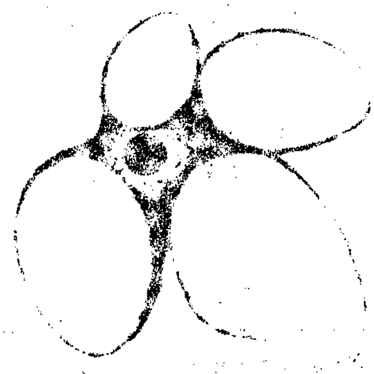


Figure 5. Section of a cell of the cortical parenchyma during intense starch formation.
The nucleus contains large, paddle-shaped starch grains. The nucleolus of the peripheral zone of the nucleus and the thin membranes of the plastid bodies are sharply pyroninophils (contain high concentrations of RNA) (X 2240)

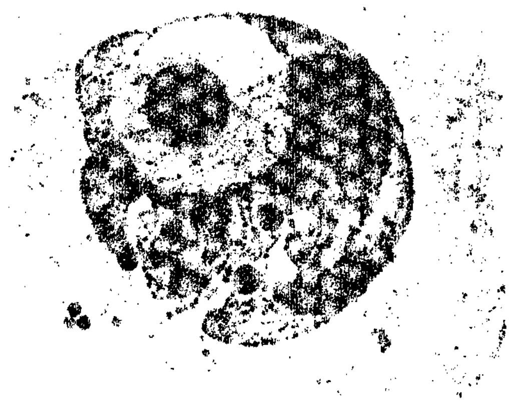
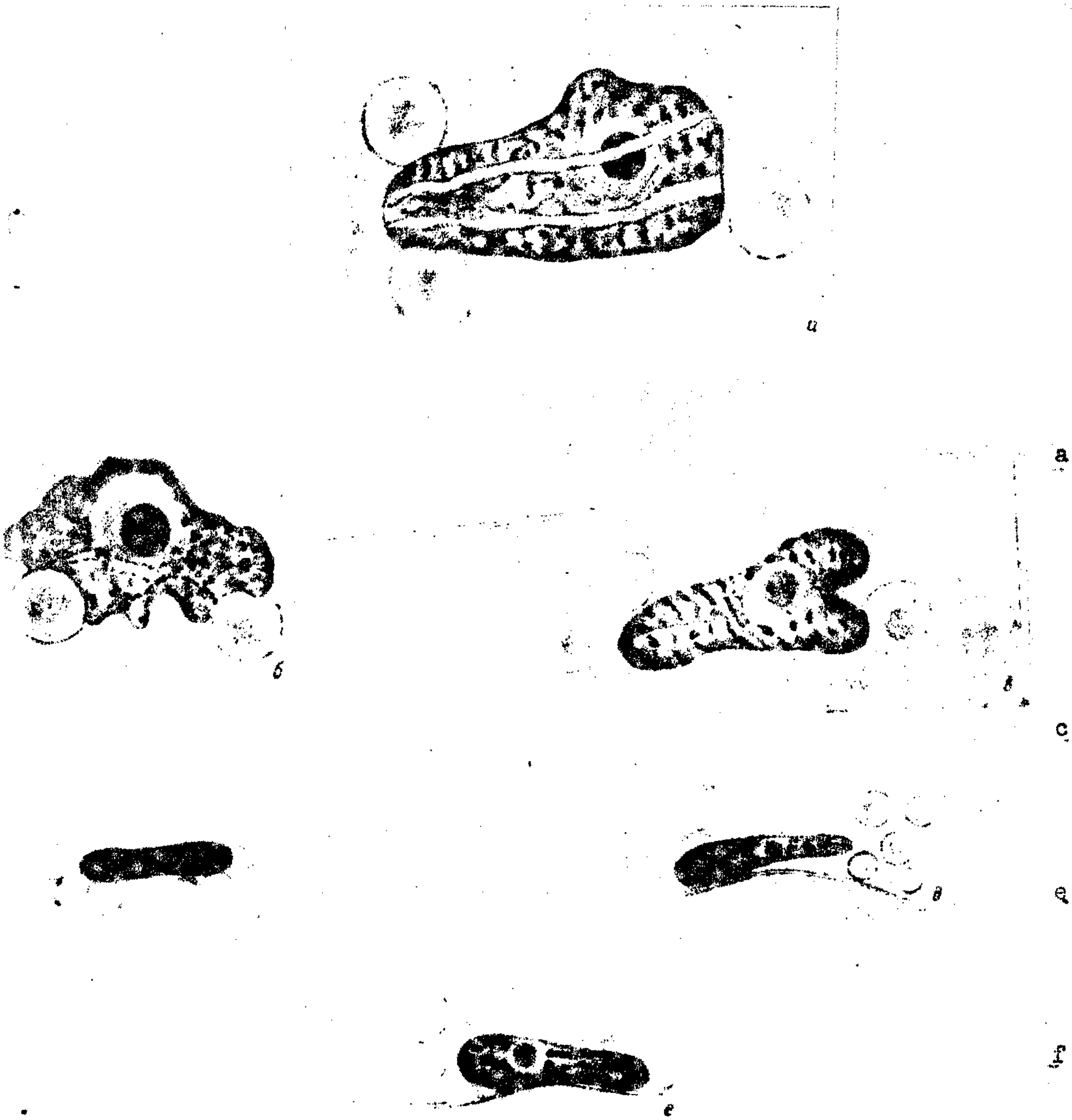


Figure 6. Nucleus after protracted functioning in a starch-forming cell (X 2240).

gure 7. Mitosis in the starch-forming cells of degenerate potato tubers.
- anaphase, the cell does not contain starch grains; b - complex metaphase in binuclear cell; c - metaphase of nucleus with an increased number of chromo-ses; d - trinuclear cell.



gure 8. The nuclei of cells of the storage parenchyma of a young degenerate ber. a, b, c - nuclei with clear-cut patterns of premature "senescence," weak velopment of starch grains; d, e, f - lengthened basophile nuclei with the st sharply suppressed starch formation (X 2240).

Figure 9. Section of a starch-forming cell from a mature tuber of a degenerate type.
The nucleus and undeveloped starch grains adjacent to it (X 2240).

THE HEAT RESISTANCE OF ACTINIA AND THEIR CILIATED EPITHELIUM UNDER NATURAL CONDITIONS AND AFTER EXPERIMENTAL CHANGES IN THE ENVIRONMENTAL TEMPERATURE

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Adaptation of the organism to the environment has always attracted the attention of biologists in different fields. Until recently, however, research workers concentrated chiefly on adaptation of the whole organism to factors in the environment, paying relatively little attention to the role of the cells in the adaptation process. Adaptation of the organism to environmental factors may be effected simultaneously by cellular as well as by organismal (systemic) mechanisms.

D. N. Nasonov's school made some attempts to compare the reactions of the organism and its cells to external influences. Thus, P. V. Makarov (1938) studied the relationship of the concentrations of narcotics which would cause general (organismal) and cellular narcosis and found that in low Protostomia these concentrations coincide, but that in higher Protostomia and Deuterostomia the entire organism was more sensitive than its cells.

In a joint report with L. N. Pisareva (1958), we showed that this pattern could not be extended to sensitivity to heat which differs for the tissue and the organism, not only in Deuterostomia and higher Protostomia, but also in the lower Protostomia. We also established that reactions of entire organisms were markedly more sensitive to high temperatures than cellular reactions.

Temperature is the most important environmental factor for cold-blooded animals. It was shown that the reaction of cells to high temperatures was relatively constant for cold-blooded animals, within the limits of one species. The heat stability of the cells of various species of cold-blooded animals is different and has been found to correspond with the temperature conditions of their habitat (Battle, 1926; Patzl, 1933; Adensammer, 1934; Aleksandrov, 1952, Lopatina, Ushakov, and Shapiro, 1953; Ushakov, 1955; 1956 a, b, 1958; Schlieper and Kowalski, 1956; Schlieper, Kowalski and Ermann, 1958).

Starting from the protein theory of stimulation of D. N. Nasonov and V. Ya. Aleksandrov (1940), one might expect that denaturized changes in the cellular proteins constitute the basis for the reaction of cells to increased temperature. In favor of this theory are data from a number of research workers which indicate that the reaction of the protoplasm to the action of high temperatures is directly connected with the thermal denaturization of isolated proteins (Battle, 1929; Mirsky, 1937; Butkevich, 1948; Romanov, 1949; Ushakov and Gasteva, 1953; Komkova and Ushakov, 1955; Ushakov, 1956 b; Braun, Nesvetayeva, and

Fizhenko, 1959). Thus, there are grounds for speaking of protein adaptation as one of the mechanisms by which cold-blooded animals adapt themselves to the temperature conditions of their habitat.

Included in the purpose of this work is the study of the possibility of the adaptation of actinia to the action of increased temperature and the clarification of the question as to the level--cellular or organic--at which these adaptation reactions are effected.

Methodology

Actinia equina L. from the Barents Sea and the Black Sea [See note 1] served as objects of this research work. Their adaptation reactions to changes in salinity and the action of certain other stimuli were studied by us previously (Zhirmunskiy and Kiseleva, 1957; Zhirmunskiy, 1958). The sensitivity of actinia to the action of increased temperatures was studied; sensitivity was evaluated under narcosis. Reactions of entire actinia were studied in animals which had been placed in a glass jar and fastened to its bottom. Sea water of the required temperature, and held to an accuracy of 0.5 degrees, was poured into the jar. The time under narcosis included the interval of time from the beginning of the action of high temperature to the moment of complete loss of visible contractile reactions, both spontaneous [see note 2] and in response to being touched by a glass rod. The mean time under narcosis, obtained from experiments with 5-10 actinia, was determined for each temperature [level].

(Note 1] Zoologists regard Barents Sea and Black Sea actinia, which were used in the experiments described above, as belonging to the one species Actinia equina (Rakh, 1914 a, b; Zernov, 1914; Deryugin, 1915; Kuznetsov and Matveyeva, 1948; Prokudina, 1952, and others.)

(Note 2) The mechanisms of the so-called spontaneous contractile reactions of actinia and other anchored sea animals has aroused the interest of many research workers in recent years (refer, for example, to Pora and Nitu, 1948; Koshtoyants and Smirnova, 1955; Ross, 1955).

Semilogarithmic graphs were constructed to depict the dependence of the time under narcosis upon the temperature. Temperature was plotted along the x-axis and the time under narcosis (logarithmic scale) was plotted along the y-axis. With such a graph the time under narcosis is an exponential function of the temperature.

Ciliary epithelium from actinia septa whose thermal sensitivity could be judged by the time of thermonarcosis were selected as the objects of this study of cellular reactions. To make the preparations the middle portion (without the foot, and the mouth opening, which is surrounded with tentacles) is cut from the body of the actinia, and this portion is then divided into several sectors. A thread is passed

through the exterior part of a sector and the sector is tied to a glass rod. On the interior part of the sector are the mesenteric filaments of the septa, covered with ciliary epithelium. Glass rods with pieces from 6-8 actinia are lowered into a thermos filled with heated sea water (the temperature of the water varies within limits of - 0.2 degrees). Then the rods are periodically removed from the thermos, a small bit of ciliary epithelium is cut from each piece and examined under a microscope.

Control experiments showed that the reaction of ciliary epithelium separated from the ectodermal part of a sector to the action of high temperatures does not differ from the reaction of the epithelium on a piece of actinia. Therefore, pieces (sectors of actinia) separated by the method described above were used in the experiments. The time of narcosis of the ciliary epithelium was defined to be the interval from the beginning of the action of high temperature to the moment its cilia ceased to move. As was done in the case of whole actinia, curves were constructed showing the time of narcosis to be a function of temperature. The experiments were conducted in June 1958 in the Murmansk Biological Station of the AN, SSSR and in September of the same year in the Karadag Biological Station of the AN, SSSR. Part of the experiments were accomplished jointly with T. A. Shlyakhter.

Results

The results of the experiments are presented in the table and in Figure 1 - 4. In Figure 1 are shown the curves of thermonarcosis for whole Barents Sea and Black Sea actinia contained in sea water, the temperature of which differed hardly at all from the temperature of the water in the sea (5-7 degrees for northern and 19-22 degrees for southern actinia). These curves were obtained two times--in 1949 and 1952, and in 1958. As may be seen from the figure, only one straight line apiece can be drawn through the points obtained in previous and in new experiments on both types of actinia. That is, these data practically coincide. The curve of the dependence of the time of narcosis upon the temperature for Barents Sea actinia is to the left of that for Black Sea actinia; that is, the Barents Sea actinia were more sensitive to the action of higher temperature than were the Black Sea actinia.

Object and Temperature at Which the Actinia Were Maintained	The Temperature of Reaction (In Degrees Centigrade)													
	24	26	28	29	30	32	34	36	37	38	39	40	41	42
Barents Sea actinia, 5-7	80	25	13	9.6	2.8	-	-	-	-	-	-	-	-	-
Barents Sea actinia, 15-20	-	194	30.4	12	6.7	-	-	-	-	-	-	-	-	-
Ciliary epithelium of Barents Sea actinia 5-7	-	-	-	-	149	65	7	3.2	-	1.3	-	-	-	-
Ciliary epithelium of Barents Sea actinia, 15-20	-	-	-	-	156	62	11.2	3.2	-	1.5	-	-	-	-
Black Sea actinia, 19-22	-	-	-	-	-	-	51	14.8	-	7	-	1.6	-	-
Ciliary epithelium of Black Sea actinia, 19-22	-	-	-	-	-	-	-	-	105	49	32	8.9	4.2	2

The difference in sensitivity of the northern and the southern actinia were revealed most vividly by comparing the time of narcosis of the specimens at the same temperature. At 30 degrees it amounted to about 5 and 800 minutes respectively, that is, whole Barents Sea actinia were narcotized 160 times more rapidly than Black Sea specimens.

The curves of heat stability of isolated ciliary epithelium of the septa of Barents Sea and Black Sea actinia are shown in Figure 2. Here the same response to the action of high temperature as that seen in whole animals is maintained, even though the difference in time of narcotization is somewhat less (47 times at 38 degrees). [See note].

[Note] It must be noted that in both cases, the organic [reflex] reactions are more sensitive to the action of high temperature than cellular reactions [Figure 3], which agrees with the data obtained previously with other marine nonvertebrates [Zhimunskiy and Pisareva, 1958].

The difference in thermal sensitivity of actinia can also be evaluated by the inverse method--by noting temperatures which lead to narcosis for the same time. For ten minutes, for example, whole Barents Sea actinia (Figure 1) were narcotized at 28 degrees, and Black Sea actinia at 37 degrees, that is, the difference in their thermal sensitivity amounted to 9 degrees. In like manner, the ciliary epithelium (Figure 2) were narcotized at 34 degrees and at 40 degrees, that is, the difference amounted to 6 degrees. It is interesting to contrast these results with the temperatures prevailing in the habitats of the actinia. According to data from literature, the maximum water temperatures in the coastal zone is 11 degrees for the Barents Sea and 27 degrees for the Black Sea (Voronkov, Uralov, and Chernovskaya, 1948; Zernov, 1914). Consequently the difference between them amounts to 16 degrees.

Thus, the difference in thermal sensitivity of northern and southern actinia depends directly upon the difference in the temperature conditions of their habitat. The difference in thermal sensitivity is carried over to the cellular level, which permits one to speak of the presence in this case of both organic as well as cellular adaptive reactions.

It has been shown that actinia possess great plasticity in respect to changes in salinity. Thus, when subjected to gradual changes in the salt concentration of the sea water in which they are kept, Black Sea actinia accommodate themselves in the course of a few days to concentrations twice the original value (Zhirmunskiy and Kiseleva, 1957). In Pax' experiments (Pax, 1910) Metridium schillerianum actinia survived gradual freshening of the water up to 4°/00. Finally, M. I. Kiseleva and I succeeded in "training" Barents Sea actinia, brought to the Sevastopol' Biological Station, to Black Sea water the salinity of which is but little more than half that of the Barents Sea. These actinia have lived at Sevastopol' more than six months now and a portion of them have produced descendants.

Starting from these data, it seemed tempting to us to try to "train" actinia to sea water at higher temperatures. The experiments were set up at the Murmansk Biological Station. Along with the control group of actinia which were maintained at 5-7 degrees, a portion of the actinia were placed in sea water whose temperature fluctuated from 15 to 20 degrees. These actinia were under observation for ten days, during which time the animals did not differ from the control ones in respect to external appearance and behavior. Then experiments were set up for the actinia which had been in the warm water for seventeen days to study their thermal sensitivity. The results of these experiments are given in Figure 4. It is obvious from the figure that the thermonarcosis curve for actinia kept in warm water is markedly shifted to the right as compared with the curve for actinia from cold water. This means that the actinia which were kept in warm water had become markedly more stable to the action of increased temperature than those found in cold water. At 30 degrees the time of narcosis amounted to approximately 4 minutes for the first and 11 minutes for the second. Thus, we succeeded in increasing the stability of actinia to temperature more than two fold under experimental conditions.

Further, it seemed interesting to determine the level--cellular or organic--at which the adaptation of the actinia to increased temperature noted in these experiments had occurred. To solve this problem, experiments were set up to compare the thermal stability of isolated ciliary epithelium of actinia kept at 5-7 degrees and at 15-20 degrees. The points through which the general curve (Curve 3 of Figure 4) for the ciliary epithelium of actinia kept both in cold and in warm water is constructed, constitutes the result of these experiments.

Thus, the increased thermal stability revealed in the experiments described here of actinia kept in warm water is organic adaptation.

The data set forth here harmonize with a large number of studies showing that the cells of cold-blooded animals are characterized by a marked conservativeness of reactions to the action of high temperatures (for sources in literature, refer to Ushakov, 1958). Moreover, it is interesting to compare our data with the result of research conducted by Yu. I. Polyanskiy (1957) in studying the temperature adaptations of Protozoa. Yu. I. Polyanskiy showed that, in contrast to the tissue cells of multicelled animals, freely moving unicellular animals (Infusoria) can change within a few hours their thermal sensitivity, depending upon the temperature at which they are kept. Thus, the reactions of Infusoria to high temperature proceeds in a manner analogous to the reactions of whole actinia, not to their tissue cells.

Conclusions

1. Experiments on Actinia equina L. from the Barents Sea and the Black Sea showed that the stability to high temperature is higher in Black Sea actinia than in Barents Sea specimens, which is in direct dependence upon the difference in the temperature conditions of their habitats.

2. An analogous difference in thermal stability was also revealed in isolated ciliary epithelium of actinia, which permits one to speak of the presence, in this case, of both organic and cellular adaptive reactions.

3. When Barents Sea actinia were kept in warm water for seven-ten days, it was possible experimentally to increase their thermal stability more than two-fold. At the same time the thermal sensitivity of their ciliary epithelium did not change.

4. The data obtained show that the adaptation of actinia to high temperature is accomplished both through organic as well as through cellular mechanisms. At the same time, the cellular adaptations are characterized by an incomparably greater conservatism than the organic adaptations.

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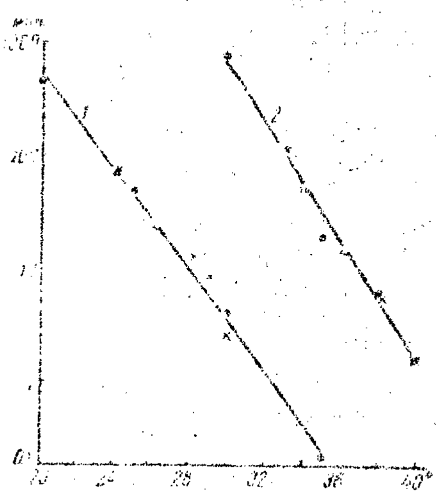
FIGURE APPENDIX

Minutes
1000



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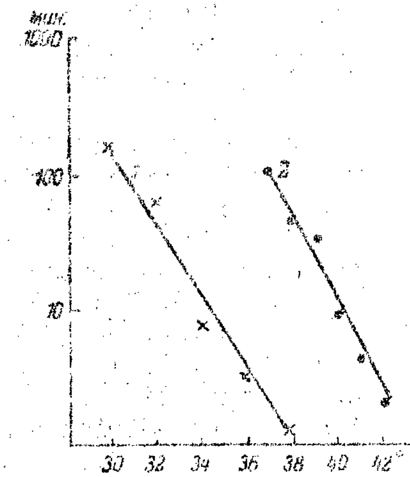

are 1. Dependence of time of
otization (in minutes of
nts Sea (1) and Black Sea (2)
inia upon temperature (in degrees
tigrade).
of 1949 are shown by dots on
1, the data of 1952 by dots on curve 2;
data of 1958 are shown by crosses on
curves. The scale of the ordinates is
arithmic.

Figure 2. Dependence of the time of narcotization (in minutes) of the ciliary epithelium of Barents Sea (1) and of Black Sea (2) actinia upon temperature (in degrees Centigrade).
The scale of the ordinates is logarithmic.

Minutes
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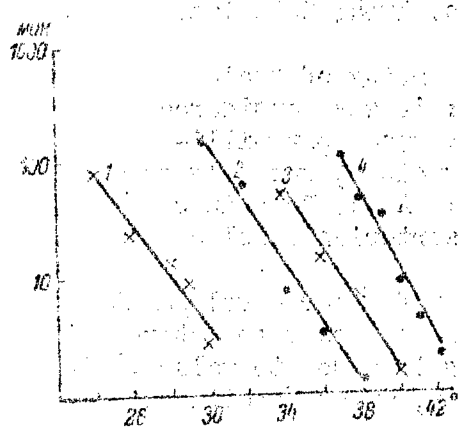

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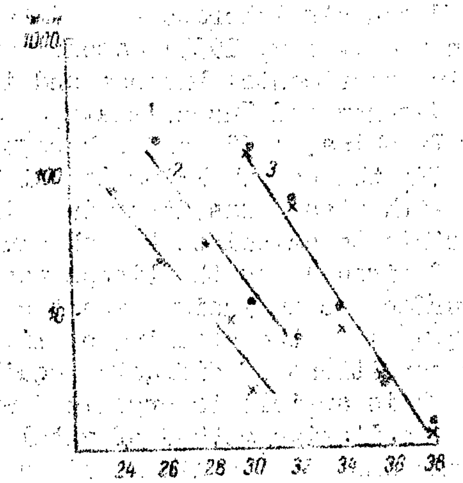

Figure 3. Dependence of time of narcotization (in minutes) of whole actinia and their ciliary epithelium upon temperature (in degrees Centigrade).
1 - whole Barents Sea actinia, 2 - ciliary epithelium of Barents Sea actinia, 3 - whole Black Sea actinia, 4 - ciliary epithelium of Black Sea actinia.
The scale of the ordinates is logarithmic.

Figure 4. Dependence of time of narcotization (in minutes) of whole Barents Sea actinia kept at 5 - 7° (1) at 15 - 20° (2), and their ciliary epithelium (3) at 5 - 7° (dots) and at 15 - 20° (crosses) upon temperature (in degrees Centigrade).
The scale of the ordinates is logarithmic.

FIXING OF PHENOL RED BY LIVE AND KILLED ANIMAL TISSUES UNDER CONDITIONS OF DIFFUSE EQUILIBRIUM

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The quantitative method of vital staining which was developed in D. N. Nasonov's laboratory to determine the functional state of tissues (Braun and Ivanov, 1933; Nasonov and Aleksandrov, 1940), is now widely used in experimental biology and in medicine (Nasonov and Ravdonik, 1947; Nasonov and Suzdal'skaya, 1953; Krasil'nikova, 1954; Golovina, 1955; Troshina, 1957, and others).

In the overwhelming majority of works the quantity of stain fixed with tissue was determined in relative units; it was considered impossible to calculate the absolute value (in micrograms per milligram of tissue), as the tissue was not completely stained, but only on the surface to an insignificant depth. We have only A. S. Troshin's data (1956) on the staining of live and dead frog sartorius muscles under conditions of diffusion equilibrium.

This article is organized with the purpose of further development of a quantitative method of vital staining. It was necessary to determine the amount of stain fixed by different tissues in absolute values. This could be done only with total staining, that is, under conditions of diffuse equilibrium.

Live and killed tissue from grass frogs sartorius muscle, kidney, and cornea) and muscle tissue from Mytilus edulis (the anterior and posterior retractors of the bissal gland) served as the objects of study. The work was done on frog tissues during the winter months and on the muscles of M. edulis during the summer months. An acid diffuse stain, phenol red in a concentration of 0.06 per cent, was selected as the vital stain. For frog tissue the staining solution was prepared on Ringer's solution and for muscles from M. edulis on sea water.

Prior to experimenting the prepared frog tissues were kept in Ringer's solution for 1. - 1.5 hours while the muscles from M. edulis were kept in sea water. The duration of staining varied from a half hour to 13 hours (up to the onset of diffuse equilibrium). Frog tissues were stained at 19 degrees and tissues from M. edulis at 23 degrees. The stain fixed by the tissue was extracted by 70 per cent acidified ethyl alcohol. The extracts obtained were subjected to colorimetry in a FEK-M photoelectric colorimeter and the stain content in them was determined with the aid of a calibrated curve, then the quantity of stain was calculated in terms of micrograms per milligram of dry tissue (arithmetic mean -- M, and its mean square error -- m). It was interesting to compare the sorption capacity of living tissues with that of

dead tissues. The tissues were killed in several ways: 1) the action of Ringer's solution, heated to 80 degrees, for a duration of 5 minutes; 2) the action of distilled water, heated to 80 degrees, for a duration of 5 minutes; 3) the action of 70 per cent ethyl alcohol for a duration of 30 minutes to one hour, depending upon the thickness of the tissue. When the first and the third methods were used, frog tissues were soaked in Ringer's solution and tissues from M. edulis were soaked in sea water for 21 hours prior to staining.

The data obtained from staining live frog sartorius muscles are presented in the figure. The duration of staining (in hours) is plotted along the x-axis and the quantity of phenol red fixed per milligram of dry tissue (in micrograms) is plotted along the y-axis. Each point of the curve is the mean of 10 experiments.

As the figure shows, diffuse equilibrium is established in 4 hours; by this time 1 milligram of dry tissue has fixed 0.88 micrograms of phenol red. These results correspond to Troshin's data (1956) in whose experiment 1 milligram of dry tissue fixed 1 microgram of phenol red. Every tissue studied by us yielded the very same type of curve, like that shown in the figure. On this basis a table was set up which showed the time for the onset of diffuse equilibrium (in hours) and the amount of stain fixed by the tissue during this time.

The Quantity of Phenol Red Fixed by Different Tissues under Conditions of Diffuse Equilibrium (in Micrograms Per Milligram of Dry Tissue) and Times for the Onset of This Equilibrium (in Hours). Concentration of Stain - 0.06 per cent

Object	Live Tissue		Tissue Killed by					
			Distilled Water at 80 Degrees		Ringer's Solution at 80 degrees		70 Per Cent Alcohol	
	Time of Onset of Equilibrium (in hours)	Amount of Stain (in Micrograms Per Milligram of Dry Tissue)	Time of Onset of Equilibrium (in Hours)	Amount of Stain (in Micrograms Per Milligram of Dry Tissue)	Time of Onset of Equilibrium (in Hours)	Amount of Stain (in Micrograms Per Milligram of Dry Tissue)	Time of Onset of Equilibrium (in Hours)	Amount of Stain (in Micrograms Per Milligram of Dry Tissue)
Sartorius muscle of frogs	4	0.88+0.05	10-13	14.9+0.3	5	24.7+2.3	6	27.6+0.4
Frog kidneys	3	2.46±0.17	1.5	4.9±0.2	5	27.6±1.6	5	44.3±2.5
Frog corneas	1.5	1.6 ±0.21	1.5	26.3±1.4	2	21.9±1.4	3.5	4.7±0.6
Mytilus anterior retractor	2.5	1.3±0.17	-	-	3.5	14.8±0.8	2-3	9.7±0.3
Mytilus posterior retractor	5	1.6±0.06	-	-	5	7.68±0.43	4	7.8±0.3

It follows from the data presented in the table that the times of the onset of diffuse equilibrium vary with the thickness of the tissues. The amount of stain fixed by different animal tissues varied from 0.88 to 2.46 micrograms per milligram of dry tissue. Dead tissue fixed many times this amount of stain--from 4.7 to 44.3 micrograms per milligram of dry tissue. At the same time, it is important to note that the method used for killing tissues affected their capacity to fix the stain. Thus, sartorius muscle killed by the action of heated distilled water fixed 14.9 micrograms while that killed by alcohol fixed 27.6 micrograms. Still greater differences were observed in the corneas and the kidneys. In view of this, when studying the sorption capacity of dead tissues, it is essential to indicate the method for killing the tissues.

Conclusions

1. The fixing of phenol red (0.06 per cent) of live and killed tissues from grass frogs (sartorius muscles, kidneys, and corneas) and the sea mussel Mytilus edulis (the anterior and posterior retractors of the byssal gland) was studied for different periods of staining.
2. Diffuse equilibrium for live tissues occurred, on the average, in 2-4 hours. Diffuse equilibrium for dead tissues occurred in two-ten hours.
3. At the moment of diffuse equilibrium living tissue fixed, on the average, from 0.88 to 2.46 micrograms of stain per milligram of dry weight.
4. Under conditions of diffuse equilibrium, dead protoplasm in the tissues sorbed 20-30 times as much stain as live [protoplasm].
5. The sorption capacity of dead tissue depends upon which agent was used for killing the tissue.

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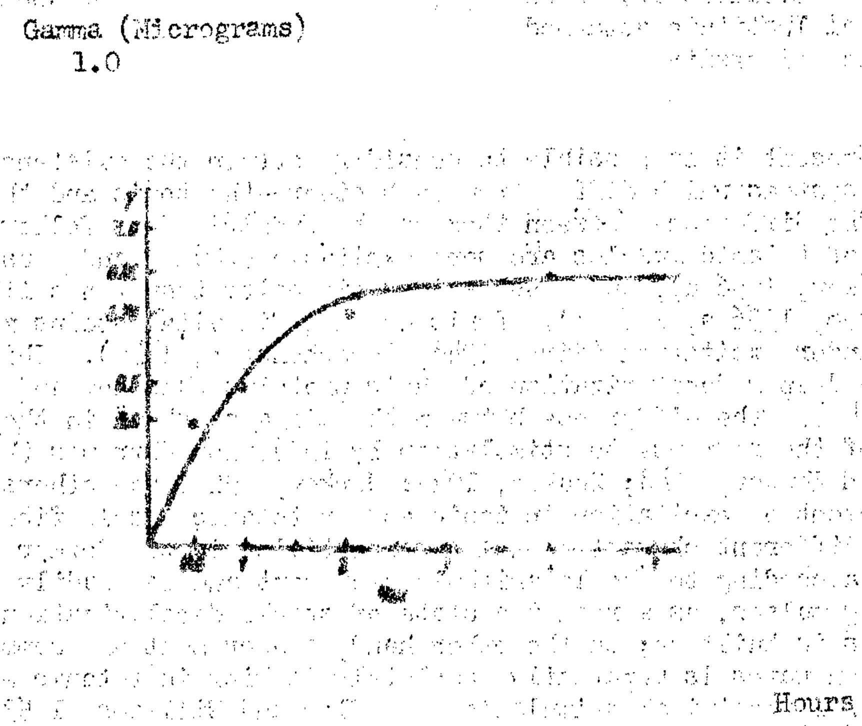
FIGURE APPENDIX



Amount of phenol red absorbed by living frog sartorius muscles in different times of staining in a 0.06 per cent solution of the stain (in micrograms per milligram of dry tissue).

ACCOMMODATION POWER OF TONIC AND TETANIC MUSCLES AND SINGLE MUSCLE FIBERS FROM FROGS

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At present it is possible to consider certain the existence of two muscle systems which differ from each other--the tonic and the tetanic. The difference between them can be summarized as follows. The fibers of tetanic muscles are more excitable (Zhukov and Leushina, 1948 a; Zhukov, 1956 a), and possess less viscosity than tonic fibers (Zhukov, 1936, 1956 a, 1956 b). Tonic muscles fix vital stains more intensely under excitation (Kiro, 1948; Vereshchagin, 1949). This indicates a deeper denaturization of their proteins (Nasonov and Aleksandrov, 1940). The difference between them is also shown in the character of the response to stimulation by induction currents (Vereshchagin and Zhukov, 1948; Zhukov, 1948; Serkov, 1948, and others). The development of excitation in tonic and in tetanic muscle fibers acquires a different character: electronegativity in the former is propagated according to the logarithmic decrement and is readily summed with leading pulses, as a result a state of stable depolarization of tonic fibers is built up; on the other hand, a decrement and summation of excitation waves is apparently completely lacking in tetanic muscle fibers under supraliminal stimulation (Kuffler and Williams, 1953 a; Zhukov, 1956 a).

Taking into consideration the difference between tetanic and tonic muscles, it is possible to make the assumption that the speed of accommodation in them is likewise not the same and that the ability of tonic fibers to maintain a state of protracted, stable excitation is partially explained by their lower accommodation power. A number of facts bear evidence in favor of this hypothesis. For example, Kuffler and Williams (1953 b) showed that under prolonged stimulation with direct current the tonic fibers from the ilioperoneal muscle had a greater capacity for maintaining the tension set up in them (the experiments were conducted in an isometric regimen of work of the muscles) than the tetanic fibers. It was shown further (Khayenko, 1952) that when subjected to lesion, tonic muscles readily fell into prolonged contracture while tetanic muscles rapidly "adapted" to the action of the lesional agent.

The facts set forth bear only indirect witness to the theory that tonic muscle fibers possess less accommodation power. Direct proof of this hypothesis is the purpose of this work.

Methodology

The experiments were conducted upon fall and winter grass frogs (males and females). The rectus abdominis, also the sartorius and PODVZDOSHNO-MALOBERTSOVAYA muscles were used in these experiments. Single muscle fibers were separated from the latter two. After preparation, the muscles and single muscle fibers were kept in Ringer's solution for not less than one hour at a temperature of 15-18 degrees Centigrade. Control experiments were conducted on curarized frogs.

Stimulation was accomplished with the aid of nonpolarizing brush Du Bois-Reymond electrodes. Some inconvenience in working with them forced us to make occasional use of silver and chlorinated electrodes, which did not affect the results of the experiments. According to Latmanizova (1949), the use of the latter electrodes has practically no effect on the results of measuring accommodation power. The distance between electrodes was 1 centimeter. A device suggested by Solandt (1935, 1936) was used to measure the constant of accommodation λ. The constant λ was calculated in accordance with the formula:

$$\lambda = \frac{RC}{E/E_0 - 1}$$

where R is a constant equal to 0.5 milliohms.

Contractions of single muscle fibers were recorded by a method described by Makarov (1939, 1947).

The data obtained from each series of measurements were subjected to statistical processing. The arithmetical mean M and the square error of the arithmetical mean m were determined. We defined the probability of differences between the arithmetical means of two series to be the quantity t, which is equal to the ratio of the value of the difference to its error:

$$t = \frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}}$$

The difference is considered trustworthy if $t > 3$. A total of 152 experiments was conducted.

Results

First, the accommodation power of the sartorius muscle and the rectus abdominis muscle were compared. The first contains only tetanic fibers, the second is a mixed muscle, including tonic and tetanic muscle fibers. Since fibers of the obliquus abdominis enter the rectus

abdominis from the side, the constant of accommodation was determined for that part located close to the white line. For this purpose, the lateral part of the muscle was cut off and λ was determined for its middle segments. According to data from Krueger and his co-workers (Krueger, Duspiva, and Fuerlinger, 1933), the tonic fibers of the rectus abdominis are located chiefly near the surface. This makes it possible to obtain an isolated response from the tonic fibers when the entire muscle is stimulated, a thing which can be judged by the character of its contraction. The response of the rectus abdominis to stimulation was a markedly slow contraction of the segment under study while the response of the sartorius muscle was rapid twitching.

Curves of the speed of accommodation for these muscles are given in Figure 1. The tonic fibers contained in the rectus abdominis are characterized by a larger value of the constant of accommodation than the tetanic sartorius muscle. The difference between them should be recognized as trustworthy as t is markedly larger than 3 (refer to the table).

Value of the Constant λ for Different Muscles and Single Frog Muscle Fibers

Object	Number of Determinations	(M + m) Milliseconds [see note]	t
Sartorius muscle	17	420+ 17)	89
Rectus abdominis	13	2300± 12)	
PODVZDOSHNO-MALOBERTSOVAYA Muscle			
a) The entire muscle	16	99+ 2	
b) "Tetanic" bundle	26	99± 3)	22
c) "Tonic" bundle	36	166± 1)	
d) Tetanic fibers	16	130± 9)	41
e) Tonic fibers	23	500± 1)	

([Note] The values of λ given here were measured with a doubled resistance base[λ = RC]).

The second stage in this work consisted of determining the accommodation power of the Ilioperoneal muscle. It is well known that this muscle consists of two parts: a lateral part which includes, basically, tetanic fibers and a central part, more highly pigmented, containing chiefly tonic fibers (Zhukov, 1956 a). Thus, it was interesting to measure and compare the λ of the entire muscles, also of their component bundles and single muscle fibers.

It was possible to separate both tonic and atonic fibers from the lateral and the central parts of the ilioperoneal muscle. In order to determine the type of muscle fiber, their contractions were recorded on film (Figure 2, a, b) after which the constant of accommodation was determined. The results of the experiments (the table and Figure 3) provide evidence that the tonic muscle elements of the ilioperoneal muscle possess less accommodation power than the tetanic. The different speed of accommodation of tonic and tetanic fibers cannot be explained by stating that the stimulus is directed in the region of their myoneural apparatuses, which then determined this divergence. Control experiments conducted on fibers taken from the muscles of frogs which had been subjected to curarization yielded identical results. The fact that the constant λ of a "tetanic" bundle from the ilioperoneal muscle has a value equal to the λ for the whole muscle is interesting. This is explained, probably, by the greater excitability of tetanic fibers (Zhukov and Leushina, 1948 a; Zhukov, 1956 a) and are therefore the first to respond to stimulation. When measuring λ for the whole muscle we obtain the value characteristic of the more excitable tetanic fibers.

Literature contains indications (Zhukov and Leushina, 1948 b; Zhukov, 1956 a) of the presence of unspecialized muscle fibers which can produce both rapid phase contractions and contractions of the tonic type. Tentative study of the accommodation power of such "transitional" fibers separated from a tonic bundle of the ilioperoneal muscle (Figure 2 c) showed that the value of λ characteristic of them varies within wide limits (150-350 milliseconds), occupying an intermediate position between the mean values for tonic and tetanic fibers (5 experiments). It is very probable that the smaller value of λ for the tonic bundle of the ilioperoneal muscle, as compared to those of single tonic fibers separated from it (refer to table), is explained by stating that when the bundle is stimulated the "transitional" fibers are first to be excited, which then determine the value of λ.

I wish to express my deep gratitude to my leader in science, Professor Ye. K. Zhukov for the assigned subject and his constant guidance.

Conclusions

1. The tonic muscles and single muscle fibers of frogs possess a lower speed of accommodation than the tetanic.

2. The low speed of accommodation may be one of the reasons why tonic fibers are able to maintain a prolonged, stable excitation.

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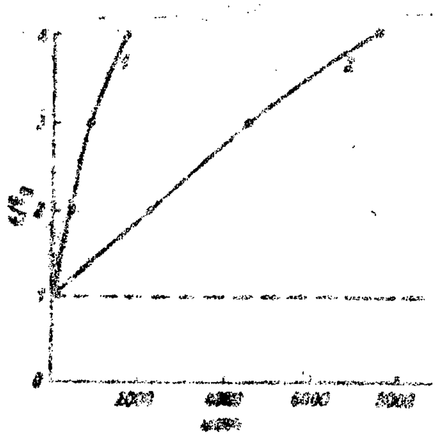
FIGURE APPENDIX

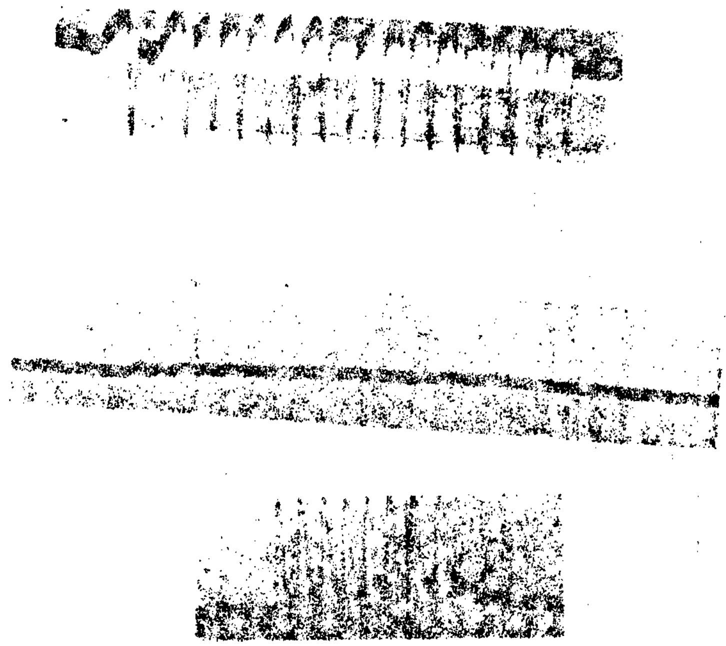


E/B 1

0

2000 Milliseconds 8000

Figure 1. Curves showing the rate of accommodation of the sartorius muscle (1) and the rectus muscle (2) of a frog.
The abscissae show the time for increase in the stimulating current; the ordinates show the threshold power of stimulating (in units which are multiples of the basic resistance). The dots represent arithmetic means of several measurements.

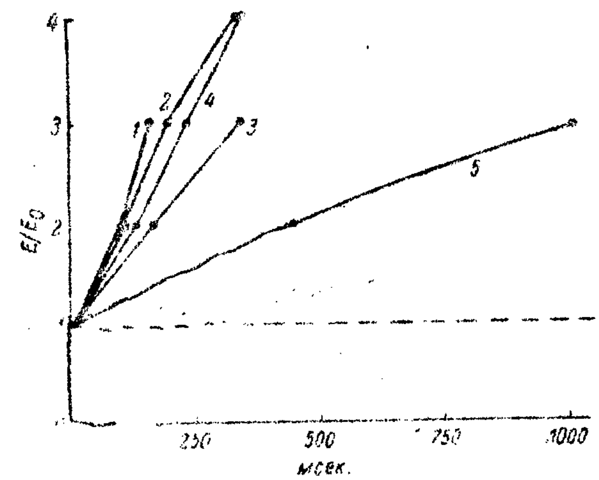


a

b

c

Figure 2. Myograms of contractions of single frog muscle fibers. a - contraction of a tetanic fiber. Stimulation by induction current with frequency of 4 cycles per second. Threshold stimulation with 17 centimeter separation of DuBois-Reymond coils. λ = 135 milliseconds. b - contraction of a tonic fiber. Same conditions of stimulation. Threshold power at 14 centimeters. λ = 500 milliseconds. c - contraction of a "transitional" fiber. Same conditions of stimulation. Threshold power at 14 centimeters. λ = 250 milliseconds.



250 Milliseconds 1000

Figure 3. Curves of rate of accommodation of the PODVZDCSHNO-MALOBERTSOVAYA muscle and its component muscular elements. 1. Ilioperoneal muscle, 2 - "tetanic" bundle, 3 - "tonic" bundle, 4 - tetanic muscle fibers, 5 - tonic muscle fibers. The coordinate axes are the same as in Figure 1. Dots represent arithmetic mean of several measurements.

EFFECT OF ALKALI METAL CHLORIDES ON THE QUIESCENT CURRENT FROM THE SKELETAL MUSCLE OF FROGS

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According to the phase theory of bioelectrical potentials (Nasonov and Aleksandrov, 1944), the difference in potential between an incision and an intact piece of tissue is the sum of two jumps in potential-- at the boundary of the damaged and the undamaged pieces and at the place of contact of the liquid electrode with the intact surface. In this connection, the potential difference should change not only with different influences acting on the intact surface, but also with the action on a transverse incision. Considerable data in the literature favor this. Thus, the change in the potential difference which occurs when temperature acts on the damaged and the undamaged regions of a nerve was discovered by Verzar (1911); on muscle by Pauli and Matula (1916). Steinbach (1933) showed that the potential difference changed more strongly under the action of sea water, solutions of the chloride salts of calcium, magnesium, sodium, potassium upon the surface of incisions in scallop muscle than when these same salts were acting on an undamaged surface. Krouse and Burge (1936) observed the change in potential difference when salts acted on a transverse incision in the gastrocnemius muscle of a frog. Placing drops of $CaCl_2$ on the incision destroyed the potential difference, and a subsequent application of Na_2HPO_4 restored it.

This work is a study of the relationship between the value of the potential difference and the action of isonormal solutions of the chloride salts potassium, rubidium, ammonia, cesium, sodium, and lithium on transverse incisions made in frog sartorius muscles.

Methodology

The experiments were conducted upon the sartorius muscles of grass frogs in the autumn of 1958. The prepared muscles were kept in Ringer's solution for 1.5 hours, after which the pelvic end of the muscle was cut off with a sharp razor in such a manner that the length of the remaining portion was 2 centimeters. Then the muscles were transferred into a moist chamber where they were suspended in such a way that the surface of their incisions only made contact with the Ringer solution, and the first measurement of the potential difference was made immediately (not more than one minute from the instant the incision was made). After

this Ringer's solution was replaced (in the experiment) by solutions of the salts being tested. Their concentration was 0.76 normal; they were prepared on Ringer's solution. The experimental series of observations were always paralleled by control observations in which the incision was in contact with Ringer's solution only (control) throughout the entire period. Later the potential difference was measured in both series every 5 - 10 minutes for an hour.

The potential difference was picked up by nonpolarizing calomel electrodes with agar faucets (3 per cent agar in Ringer's solution). One of the electrodes was placed on the intact surface of the muscle and the other in the solution. The potential was measured by a balancing method. A mirror galvanometer served as a null instrument. A millivoltmeter was used as the measuring instrument. The experiments were conducted at a room temperature of 15-17 degrees.

Results

The experimental data present graphically in Figure 2 show that the potential difference in the control and the experimental muscles increased for 10-15 minutes after making the incision and this increase was always more marked in the experimental series than in the control. The maximum potential difference was reached in 15 minutes, a level which was held for 10-15 minutes for the experimental muscles and 20-30 minutes for the control muscles, after which it dropped slowly.

Mean values of the potential difference are given in the table one minute and 15 minutes after the incision was brought into contact with the solution. The last two columns of the table give the increase in the potential difference in 15 minutes. In this case, the increase in the control, 3 millivolts, is taken to be 100 per cent and the increases in potential difference in all other cases (of the table) are calculated in percentages of this value.

Changes in the Potential Difference of Frog Sartorius Muscles When a Transverse Incision is Acted upon by Chlorides of the Alkali Metals

Electrolite	Number of Experiments	Potential Difference in Millivolts		Increase in Potential Difference in 15 Minutes	
		In 1 Minute $M \pm m$	In 15 Minutes $M \pm m$	In Millivolts	In % of the Control
Ringer's solution (control)	52	30 ± 0.84	33 ± 1.39	3	100
LiCl	8	27 ∓ 1.3	37 ∓ 1.97	10	330
NaCl	8	28 ∓ 1.26	41 ∓ 1.64	13	435
CsCl	10	30 ∓ 0.6	44 ∓ 1.36	14	465
NH_4Cl	8	30 ∓ 1.28	44 ∓ 2.28	14	465
RbCl	10	29 ∓ 0.22	46 ∓ 1.34	17	570
KCl	8	28 ∓ 2.03	46 ∓ 1.64	18	600

The results showed that when the tested salts acted upon the incisions more marked increases in the potential difference were observed than were noted in the control series. The amount of this increase depended upon the cations acting upon the incision in the muscle since the anion of all the salts was Cl^-. The greatest increase in potential difference was noted with KCl. Here the potential difference increased by 18 millivolts, that is, 6 times the increase in the control. LiCl had the least effect as the potential difference increased by 10 millivolts, or 3.3 times the increase brought about by the action of Ringer's solution.

All the tested monovalent cations can be arranged in the following series according to their influence on the potential difference:

$$--K > Rb > NH_4, Cs > Na > Li + \quad (1)$$

The increase in potential difference brought about by the action of monovalent cations on transverse incisions in muscles is, as one might think, the consequence of phase and diffuse potentials occurring on the boundary between the damaged and the undamaged regions. The cations are arranged in the following series according to the values of the diffuse potentials (the calculations were carried out in accordance with Henderson's formula (1907)):

$$--Cs > Rb > K > NH_4 > Na > Li + \quad (2)$$

This series differs from (1) as Cs and Rb have been shifted. Therefore, it is possible to think that the shifting is connected with their influence on the phase potential.

Series (1) obtained from experiment coincides wholly with the series obtained by Hoeber (1905), Seo (1927), Nasonov and Aleksandrov (1944), Troshing (1948), Golovina (1948) in their studies of the effects of currents derived from salts on muscle. This coincidence is understandable if one considers that the mechanisms which set up currents in salts and in damaged tissue do not differ fundamentally.

The fact that the potential difference changes when salts act on an incision provides evidence that the potential difference depends upon processes which are taking place within the region of damage. The very same conclusion was drawn by Steinbach (1933) on the basis of experiments on scallop muscles.

Conclusions

1. When chlorides of alkali metals act on a transverse incision across the sartorius muscle of a frog, an increase is caused in the potential difference between the damaged and the intact portions of the muscle, an increase which is markedly greater than the

increase in potential difference caused by the action of Ringer's solution on the incision.

2. The potential difference increases in accordance with the arrangement of cations in the series

$$-- K > Rb > NH_4 , \quad Cs > Na > Li +$$

3. The data obtained provide evidence that the potential difference depends upon processes which are taking place within the region of damage of the muscle.

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FIGURE APPENDIX

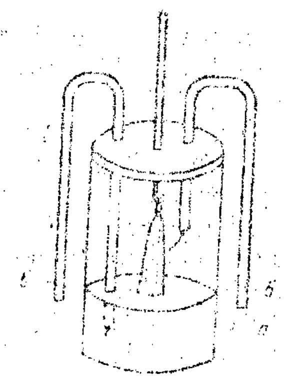
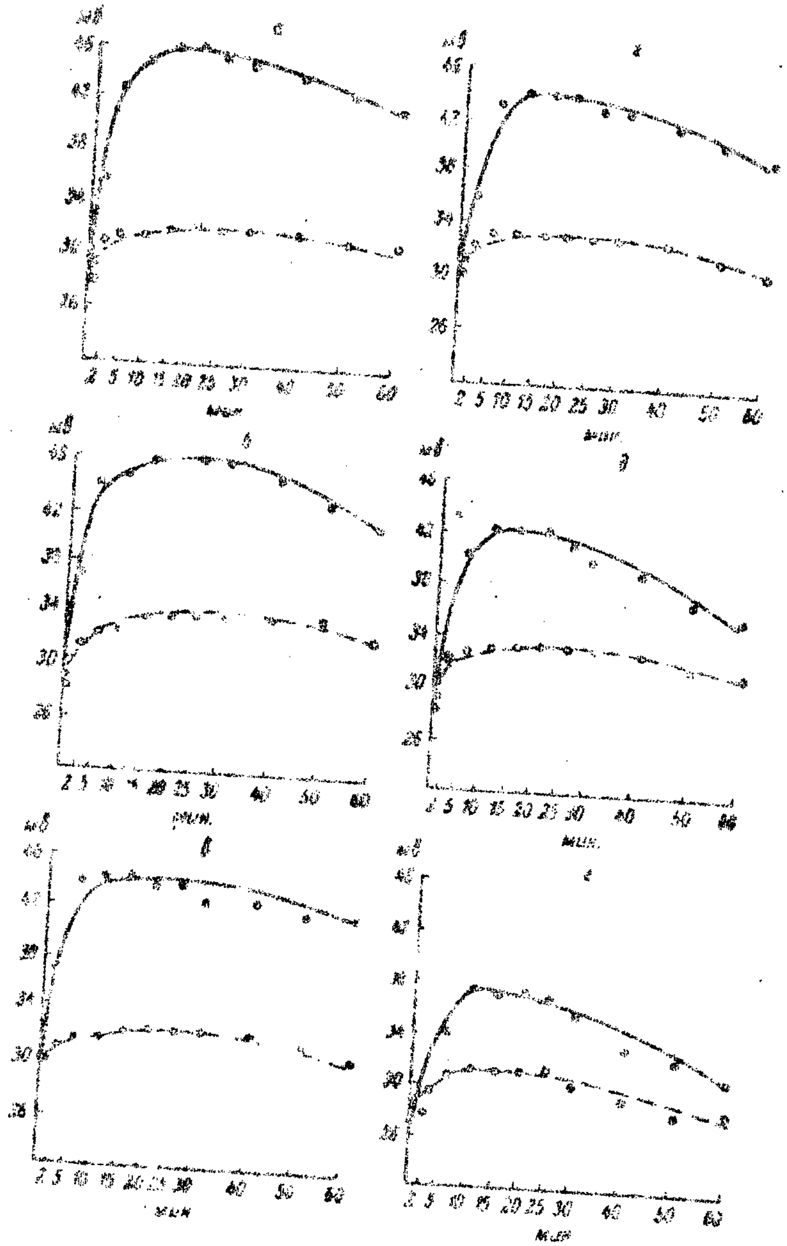


Figure 1. Chamber for picking up potential difference. a - muscle, b - pick up electrodes.



llivolts a
6
2
Minutes 60

Millivolts
46 d
26
2
Minutes 60

livolts
6 b
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Millivolts
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livolts
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2 60
Minutes

Millivolts
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Minutes

ure 2. Changes in the potential difference of frog sartorius muscles when orides of the alkali metals act on a transverse incision. a - KCl, b - RbCl, NH_4Cl, d - CsCl, e - LiCl. Solid lines - experimental series; dashed lines - rol series. Each dot represents the arithmetic mean of 8-10 measurements.

THE CHARACTERISTICS OF PROTEIN SYNTHESIS IN THE ORGANOIDS OF CELLS IN THE TISSUES OF NORMAL AND IRRADIATED WHITE RATS

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It was shown in previous studies (Il'ina, 1957, 1958; Il'ina, Blokhina and Uspenskaya, 1957) that in acute radiation sickness of rats, caused by x-rays, a change takes place in the correlation between the content of different proteins in cellular organoids and the rate of synthesis of these proteins. At the same time it was discovered that in acute radiation sickness changes also take place in the antigenic properties of the tissues of irradiated animals (Petrov and Il'in, 1956 a, 1956 b, 1957).

It seemed possible that acute radiation damage results not only in quantitative, but also in qualitative changes in the synthesis of proteins.

When tissue proteins are subjected to constant decomposition, all their component amino acids should be included in newly-forming protein particles with a definite speed as only in this case will the permanent amino acid composition of the latter be maintained, a composition characteristic of the normal equilibrium state. When other, unusual proteins are formed, when there may be derangements in the synthesis of proteins under pathological conditions, however, the speed at which the different amino acids are included will differ from the norm. Therefore, the ratio of the speeds of inclusion of merely two amino acids can, to some extent, characterize the quality of protein synthesis.

We have studied the simultaneous incorporation of two different tagged amino acids--tyrosine (C^{14}) and methionine (S^{35}).

Methodology

This work was conducted on male white rats weighing 180-210 grams which had been deprived of food for 12 hours preceding the study and received only water. In one experiment 45 rats were utilized.

Radioactive proteins in the control animals and in the animals under experiment were studied within 3.5 hours after the introduction of mixtures of solutions of radioactive tyrosine and methionine. The mixture was composed of two volumes of equal activity of solutions of C^{14} - tyrosine and S^{35} - methionine and was injected subcutaneously in the amount of 0.5 milliliters, with a strength of 0.003 microcuries per gram of live weight. The quantities of stable sulfur and carbon varied within limits of 2.6×10^{-2} to 4.9×10^{-2} micrograms.

Irradiation was done once, all of it with gamma rays from radioactive cobalt. The total dose per rat was 850 roentgens. Under such radiation all the animals died within seven days. The highest percentage of mortality was observed on the fifth day (80 per cent). The irradiated animals were studied within three days after irradiation.

The cellular nuclei were separated out by Dounce's method (1943). Isolation of the cytoplasmic microstructure--mitochrondria, microsomes, and hyaloplasm--was accomplished by the method of Hogeboom, Schneider, and Pallade (1948). Dry preparations of the proteins of the cellular microstructure were obtained by means of a method described in a previous work (Il'in, 1957). The radioactivity of the proteins was determined per 10 milligrams of air-dried preparation placed on a disk of stainless steel with a surface of 2 square centimeters. The radioactivity count was made with a B-device with an end tube.

In order to determine the relationship between the C^{14} - tyrosine and the S^{35} - methionine included in the proteins of the organoids, a double count was made of the target proteins with time intervals between them of 87 days (the half-decay period of sulfur). The necessity for double counting is explained by the fact that both tags (S^{35} and C^{14}) are β-emitters and it is impossible to determine the quantity of the first and the second by direct experimental means. Therefore we made use of the difference in their half-decay periods. In 87 days C^{14} is practically undecayed as it has a half-decay period of 5600 years. As for S^{35}, however, it has lost one-half of its activity in this time. Thus, if only tyrosine wereincluded in the protein, the results from counting 87 days later should not be changed; if only methionine were included, the results should be halved. All intermediate ratios will show a definite reduction of activity. If, for example, activity dropped to 10 per cent, this would mean that the sulfur content (S^{35}) in the preparation was 20 per cent and the carbon content (C^{14}) was 80 per cent. Consequently, knowing the initial and the final (87 days later) activity of the preparation, it is possible to determine the percent of activity caused by radioactive sulfur-methionine. This estimate is incorporated in the formula which we derived:

$$0/0\ S^{35} = 2\left\{ \frac{A_0 - A_t}{A_0} \right\} \cdot 100 \qquad (1)$$

where A_0 is the number of pulses from the protein preparation immediately after its separation. A_t is the number of pulses of the very same protein preparation 87 days later.

The preparations of radioactive proteins were kept in Petri dishes, in which pieces of cotton moistened in water had been placed to create moistness -- to avoid dispersion of powdered radioactive proteins.

Results

The data characterizing the ratio of radioactive sulfur-methionine to radioactive carbon-tyrosine in the preparations of overall proteins of the organoids of cells from normal and irradiated rats are presented in the table.

It follows from these data that when normal rats are injected with a mixture of radioactive amino acids one will observe unequal incorporation of sulfur methionine and carbon tyrosine into the protein of the organoids of the cells of the liver and the mucuous membrane of the small intestine. Thus, the radioactivity of the sulfur is 62-78 per cent, the radioactivity of the carbon is merely 38-22 per cent. Therefore, the sulfur in the methionine is incorporated in a more intense manner than the carbon in the carboxyl group of the tyrosine. It follows from the data obtained that the norm for the ratio of S^{35}/C^{14} in the summed proteins of the different components of the protoplasm of both types of tissue varies characteristically from 1.6 to 3.5. Our data correspond well with those in the literature. Thus, according to Zbarskiy and Perevoshchikova (1957), who had studied the incorporation of different tagged amino acids in the proteins of cellular nuclei and whole tissue of normal organs and malignant tumors, S^{35} - methionine is incorporated 2.9 times more rapidly than C^{14} - tyrosine into the proteins of the nuclei of the liver cells of normal rats.

In our experiments S^{35} - methionine was incorporated 3.0 and 3.2 times as rapidly as C^{14} - tyrosine into the cellular nuclei of the liver and the mucuous membrane of the small intesting of normal rats.

The ratio of S^{35} - methionine to C^{14} - tyrosine in the nuclei, mitochondria, and the hyaloplasm of the cells of the tissues under study showed practically no change with acute radiation sickness. The decrease in the value of S^{35}/C^{14} ratio observed in the proteins of the nuclei and the hyaloplasm of the cells of the mucuous membrane of the small intestine in irradiated animals cannot be considered trustworthy due to marked variations of this quantity in different experiments from the norm. However, this quantity was not below 1 in a single experiment. In the microsomes of liver cells the ratio S^{35}/C^{14} went down to 0.7 (norm of 3.4), and in the microsomes of the cells of the mucuous membrane of the small intestine down to 0.9 (norm of 1.6).

The S^{35} - Methionine Content in Preparations of the Summed Proteins in the Organoids of the Cells of Normal (N) and Irradiated (O) Rats (in Per cent) and the Ratio of S^{35} - Methionine to C^{14} - Tyrosine in These Proteins [See note.]

Organs		Number of Experiment	Number of Animals	Nucleus N	Nucleus O	Mitochondria N	Mitochondria O	Microsomes N	Microsomes O	Hyaloplasm N	Hyaloplasm O
Liver	S^{35}	1	45	81	68	74	61	79	38	78	54
		2	45	-	71	70	87	77	35	77	78
		3	45	85	79	67	70	79	43	63	80
		4	45	70	73	60	85	78	46	66	65
		5	45	66	64	75	71	70	40	78	-
		Average		75 ± 3.9	71 ± 2.8	69 ± 2.6	75 ± 4.4	77 ± 1.5	40 ± 1.7	72 ± 5.0	69 ± 2.9
	S^{35}: C^{14}			3.0	2.4	2.2	3.0	3.4	0.7	2.6	2.2
Mucuous membrane of the small intestine	S^{35}	1	45	77	60	-	80	68	51	78	62
		2	45	-	62	73	61	60	60	-	-
		3	45	79	62	70	69	69	37	78	59
		4	45	75	64	60	70	62	53	-	64
		5	45	74	61	63	70	51	32	77	64
		Average		76 ± 1.0	62 ± 0.8	67 ± 2.6	70 ± 3.0	62 ± 3.0	47 ± 1.8	78 ± 0.3	62 ± 1.4
	S^{35}: C^{14}			3.2	1.6	2.0	2.3	1.6	0.9	3.5	1.6

([Note] Standard deviation is calculated by the formula $m = \sqrt{\sum d^2}$ where $\sum d^2$ is the sum of the squares of deviations from the arithmetical mean, and n is the number of determinations.)

That there is a sharp change in the ratio of S^{35} - methionine to C^{14} - tyrosine in the proteins of the microsomes is a very interesting fact in connection with the idea that it is in these organoids of the cells that synthesis of cytoplasmic proteins is essentially localized. It was also shown in preceding research work (Il'ina, 1957; Il'ina, Blokhina, and Uspenskaya, 1957) that in acute radiation sickness, with the same length of time after exposure to radiation, the most marked deviations in the exchange of proteins was observed in the microsomes.

The data presented provide grounds for considering that in cases of acute radiation injuries ending with the death of the animals, the processes of synthesis of proteins are destroyed.

Conclusions

1. The ratio of S^{35} - methionine to C^{14} - tyrosine in the summed proteins of the organoids of cells in the liver and the mucuous membrane of the small intestine of normal rats is characterized by values of 1.6-3.5.

2. This ratio underwent practically no change in the nuclei, mitochondria, and hyaloplasm of the cells of the above-described tissues in white rats which had radiation sickness.

3. The ratio of S^{35} - methionine to C^{14} - tyrosine in the summed proteins of the microsomes of the cells of both types of tissue changed sharply with radiation sickness. In irradiated rats it was characterized by values of 0.7 in the liver and 0.9 in the mucuous membrane of the [small] intestine.

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MATERIALS FOR DESCRIBING THE EFFECT OF IONIZING RADIATION ON INDIVIDUAL DEVELOPMENT

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The purpose of this report is to present some data concerning the action mechanism of ionizing radiation on individual development.

Larvae and pupae of Drosophila melanogaster served as the material for study-normal line Alma-Ata 6 and the progeny of flies from the population of the city of Bobrov. The active factor was x-rays. The conditions of irradiation were: Coolidge tube, target distance 23 centimeters, 120 volts, 4 milliamperes, filter 1 millimeter aluminum. The intensity of radiation was 200 roentgens per minute. Doses varied from 1000 to 6000 roentgens.

Identity of age of the larvae was ensured by a short (2-3 hours) period of egg-laying and standard conditions for development: the temperature was kept at 25-0.1 degrees, the food consisted of a yeast medium with raisins and sugar. Under these conditions, pupation in control tubes was completed in 8-12 hours.

Over 100 experiments were conducted, the majority of which involved 200 to 500 experimental and control flies under observation, and in some instances, over 500 flies. Recording the changes affecting the shape and the venation of the wings, the structure of the eyes, the size and shape of the setae, et cetera, was accomplished with the aid of a binocular reading glass with a magnifying power of 20X.

A typical example of the effect of x-rays on 4-day or 5-day-old larvae is given in Table 1. A sharp difference in respect to days of emergence was observed--in the first days more or less sharply changed individuals emerged, but by the end of emergence, on the contrary, outwardly normal individuals predominated. A different result was obtained when 0-1-day pupae were irradiated (Table 2). In this case normal flies emerged first and the emergence of changed individuals was delayed.

Table 1

DISTRIBUTION OF X-RAY MUTANTS BY DAYS OF EMERGENCE IN IRRADIATED LARVAE

Conditions of Experiment	Properties of Imago	Day of Emergence First	Second	Third	Fourth	Total
Irradiation of 4-day larvae. Line Alma-Ata 6. Dose, 4000 roentgens	Norm	0	0	0	516	516
	Mutant	50	50	51	44	195
Irradiation of 5-day larvae. Population of Bobrov. Dose, 4000 roentgens	Norm	2	4	39	52	97
	Mutant	27	46	277	56	406

Table 2

DISTRIBUTION OF MUTANTS BY DAYS OF EMERGENCE IN IRRADIATED PUPAE

Conditions of Experiment	Properties of Imago	Day of Emergence First	Second	Total
Irradiation 6-24 hours after pupation. Population of Bobrov. Dose 4000 roentgens	Norm	193	14	207
	Mutant	20	178	198
Irradiation 6-24 hours after pupation. Population of Bobrov. Dose 4000 roentgens	Norm	155	138	293
	Mutant	7	159	166

The predominance of normal flies at the end of emergence and their absence or scarceness at the beginning of summer when 4-day and 5-day larvae were irradiated cannot be explained by accelerated development of the changed individuals since, on the contrary, an obvious delay in development was observed in the experimental cultures, at times a very sharp delay (a lag of 2 days compared with the control). It is impossible to explain this result by asynchronous development of the larvae prior to irradiation and lower sensitivity of larvae which had reached a lower stage of development at the time of irradiation, for when 2-day and 3-day larvae were irradiated with doses of 3000-4000 roentgens there appeared a significant percentage of mutants. There remained only one explanation--the late appearance of normal and slightly-changed individuals was connected with repair processes which had taken place within them. The possibility of repair, even in Drosophila pupae (with mechanical injuries) is evident from Lees' data (1941). Shteynberg showed with bee moth larvae that it is possible that regeneration is connected with delayed development in this case, too. Thus, the explanation offered here is not something entirely unexpected. The results obtained from irradiating young pupae agree well with this explanation. It is obvious that the process which is rapidly taking place at this time im pupae, the process of formation of imaginal organs, markedly restricts the possibility of regeneration which exist in larvae, where, for the most part, growth of the imaginal disks is taking place.

Further, we have undertaken an attempt to confirm the correctness of the repair hypothesis in the occurrence of x-ray mutants [See note]. For this purpose it was necessary to compare the direct results of irradiation revealed by studying imaginal disks, the rudiments of definite organs, with the changes recorded in individuals which had completed their development.

([Note] It is essential to note here that the possibility of repair of imaginal disks (the beginnings of imaginal organs) which had been subjected to operative intervention has been proved for the larvae of different insects (Meisenheimer, 1908; Megusar, 1908, Ubisch, 1911; Bodenstein, 1936; Shteynberg, 1938 a, b, 1939), which is confirmed by the relativity of the concepts of mosaic and regulated types of development.)

Two-day, 3-day, and 4-day larvae of the normal line Alma-Ata 6 were subjected to irradiation. The conditions of culturing and method of irradiation were the same as in the first series of experiments. For this work the "notched wing" mutation (Figure 1) and well known after the work of Goldschmidt (1935) under the name KN was selected. In addition, numerous cases in which the notches appeared on only one wing were considered as KN mutants. At the same time, other mutations were recorded.

The dorsal mesothoracic imaginal disk, from part of which the wing develops, was studied. The development of the wings in Drosophila melanogaster, studied by Chen (1929) and in particular by

Waddington (1939, 1940) is well known now and it is not specially difficult to distinguish that part of the disk from which the wing forms (before pupation it reminds one somewhat of the pinna of the human ear). The disks are fixed by Gilson's [appears to be Jilson's fixing fluid], then preparations are prepared by ordinary histological techniques. Sections are made with a thickness of 5 microns. The preparations are stained with Heidenhain's hematoxylin, and in some cases with Feulgen's [stain]. Extraction and fixation of disks from irradiated and, in parallel, from control larvae took place within 6, 12, and 24 hours after irradiation, later with one-day intervals up to the onset of the time preceding the formation of white pupae (later the wing was transformed into a long, one-layer cellular sack which was easily damaged by removal and further processing).

After irradiation the experimental and the control larvae were counted and placed in test tubes, 100 flies per tube. The number that pupated in each tube was recorded. We took larvae for making preparations from these test tubes after proper registration. Hatching was counted in every test tube and the newly-hatched flies were inspected daily.

Damage and Repair

Study of series of sections relating to experiments in which the larvae received doses of 4000 roentgens at the age of 4 days and fixation occurred in a day yielded the following results. Large masses of cellular detritus were present in the mesothoracic imaginal disks of all the preparations without exception. A considerable portion of the cells of the disk had been decomposed: the decomposition products consisted of lumps of various sizes and shapes, exceedingly sharply colored with hematoxylin. In the control disks, however, (over 100 were studied) there were no indications of destruction of cells (Figure 2a). Staining by Feulgen's [reaction] showed that nuclear material was richly represented among the decomposition products. Thus, there can be no doubt of the destructive effect of irradiation in these experiments. Nevertheless, they could not provide a solution of the problem posed since a very high mortality was observed at a dose of 4000 roentgens and an insignificant minority of the larvae reached the imago stage. Therefore, there always remains the suspicion that decomposition takes place only in the disks of larvae destined for death while the single individuals which were capable of successfully completing their development did not appear in the material subject to histological research. One cannot exclude the possibility that the disks were not injured in the larvae which reached the imago stage.

Similar results were obtained with doses of 3000 roentgens. Doses of 2000 roentgens turned out to be sufficient that the decomposition of part of the cells was obvious at a glance in the disks of all

the larvae under study (there were no deviations from the norm in the control). The material subjected to histological study (Table 3) was sufficiently large to confirm this with complete reliability. At the same time, on the average, approximately two-thirds of the irradiated individuals not only completed metamorphosis but also had normal wings. The low mortality in these experiments obviously gave rise to the comparatively high percentage of occurrences of mutants, while in the preceding experiments, with doses of 3000 roentgens, potential mutants more frequently turned out to be nonviable.) Consequently, among 272 disks under study about 180 should belong to individuals which had retained their capability to complete development and, despite injury to their wings, indistinguishable from the control flies in the imago stage. In other words, a normal wing in an individual which had received 2000 roentgens when a 4-day larva was always the result of repair of the injury received since the rudiments of the wing had been damaged in 100 per cent of the irradiated individuals.

It was of interest to investigate when, how, and where the cell destruction began and how the process of repair proceeded after irradiation with a dose of 2000 roentgens. The first lumps of decomposition were discovered in the thickness of the disks as early as 6 hours after irradiation of 4-day larvae. It is noteworthy that they appeared in the rudiments of the wing, that is, in a region of most rapid multiplication of cells, which confirms the well known rule of the connection between high sensitivity to x-rays on the part of cells and the process of cellular division.

In 12 hours a considerable portion of the cells of the disk had been destroyed (Figure 2b). In a day after irradiation one often observed further spread of decomposition. But, along with this, at the same time one also discovered the first indications of repair--the cellular detritus peeled off the healthy portion of the disk and a more or less clear line of demarcation appeared which separated the masses of decomposing cells from the living tissue (Figure 2c). In this stage the resemblance of the x-ray trauma in the Drosophila larvae to the pictures observed by Zavarzin, Yasvoyn, Aleksandrov, and Strelin (1936) in irradiated chick embryos is revealed in a particularly clear manner.

Table 3a

Irradiation of 4-Day LARVAE. DOSE 2,000 Roentgens. Fixation in a Day After Irradiation

	Disks			Wings		
Experiments	Normal	Injured	Injured (Per Cent)	Normal	KN	KN (Per Cent)
X	0	90	100	168	49	23.6
XI	0	106	100	263	46	14.9
XII	0	77	100	565	66	10.5
X + XI + XII	0	272	300	996	161	13.9

Table 3b

				Individuals with Normal Wings	
Experiments	Total of Larvae	Pupated	Reached Imago Stage	Number	Per Cent of Total Number of Larvae Taken in the Experiment
X	293	253	217	168	57.7
XI	398	377	331	263	66.0
XII	771	712	631	565	73.1
X + XI + XII	1462	1332	1179	996	68.1
Control	496	470	445	445	89.7

Later, one observes a reduction in the mass of cellular detritus in the white pupae. This does not take place through phagocytosis (we did not see phagocyte cells in any of the preparations) but obviously through resorption of the products of cellular decomposition. A comparatively small number of still retained lumps or grains of decomposition (Figure 3a) were discovered in preparations, on the edges of disks which were continuing their development.

It should be emphasized that the cellular detritus was found not only on the surface of the disk, but also inside the disk. If one were to investigate a whole series of sections from one disk, then it would always be possible to find the place where the clearly separated decomposition products would be found in one of the cavities formed by folds of the disk in the region of the "pinna" (Figure 3b). Such pictures again remind one of phenomena described by Zavarzin and others,

for example, a neural tube regenerating after irradiation, in whose lumen the elements of decomposition are preserved. The second thing which should be noted here is that there are peculiarities in the very process of restoration of the normal structure. Shteynberg (1938a, b), when analyzing his material on the restoration of the rudiments of the wing after its complete or partial removal from wax moth larvae, emphasized that this process takes place without the formation of regeneration blastema. The very same thing took place in our case, too. There occurred merely the redefinition of the role of certain cells of the future organ with further consequences following from this (multiplication, displacement, et cetera). Thus, like the restoration of the wings in operated wax moth larvae, the process we described of repair of the imaginal disks in Drosophila larvae and pupae approaches most closely not to regeneration in the narrow sense of the word, but to the phenomena of embryonic regulation.

The last stage investigated in this series of experiments was the straightening of the folds of the imaginal disk which is transformed in the white pupae into an extended stratified cellular sack with almost no cavity inside. At this stage the imaginal disk of an irradiated individual is distinguished from the control individuals by a small accumulation of lumps of decomposition products still not completely resorbed. These last remains of dead cells are always located inside the disk (Figure 3c, d).

The Significance of Times of Repair

Thus, the normal wing of individuals which were irradiated when they were 4-day larvae is always the result of repair. However, a larger or smaller number of KN mutants, characterized by notches in their wings, appear regularly in the irradiated material, along with the normal material. Even though repair does take place, it does not necessarily lead to complete restoration of the normal structure. On what does completeness of repair depend? It seems to us that one of the factors which determine it should be the factor of time. A number of indirect data argue in favor of this point of view. We formulated the repair hypothesis in connection with the difference in times of emergence between individuals with normal wings and those with the KN mutation. On the other hand, it is widely known that many mutants, including the KN, appear far more rarely when irradiated as 2-day or 3-day larvae than when older larvae are used. This difference is usually interpreted in the spirit of learning as due to "sensitive periods" (the actual sense of this term will be discussed later), but it is easy to understand it if one bears in mind that in individuals which have been subjected to some influence at an earlier age the time in which the repair process can take place is correspondingly longer. Finally, the significance of

the time factor is also made clear in Shteynberg's data (1938 a) which concern not Drosophila, but the larvae of the large bee moth, in which late removed rudiments of the wings are completely restored only if there is a delay in development.

Table 4a

Irradiation of 3-Day LARVAE. DOSE 2,000 Roentgens.
Fixation in 48 Hours after Irradiation (Experiment XIII)

Disks			Wings		
Total	Normal	Injured	Total	Normal	KN
32	0	32	195	195	0

Table 4b

Experimental Material	Total of Larvae	Pupated	Reached the Imago Stage	Individuals with Normal Wings: Number	Individuals with Normal Wings: Per Cent of Total Number of Larvae
Irradiated	300	261	195	195	65.0
Control	375	339	318	318	84.8

Experiments conducted with younger larvae confirmed our hypothesis. After irradiating 3-day larvae with a dose of 2,000 roentgens, we fixed the disks in 48 hours, immediately before pupation. Small accumulations pf cellular detritus were found in all the disks, without any exceptions, which reminded one of those remains of cell decomposition observed at later stages in white pupae when 4-day larvae were irradiated. An examination of the flies which emerged showed that all of them had normal wings (Table 4). Thus, when 3-day larvae were irradiated, repair was more effective than in 4-day larvae, and as a result, the imagos had wings of normal form. The completeness of repair was caused by its greater length of time. This result is wholly trustworthy, as the mortality in this experiment was not very high and, accordingly, the majority of the disks subjected to histological study belonged to individuals which were capable of attaining the imago stage in their development.

Changes in the Structure of the Disk

Resorption of the products of cellular decomposition always proceeds successfully. Nevertheless, part of the individuals which emerge will have notches in their wings if they had been irradiated as older larvae. In search of identifying signs which permit one to distinguish these cases of incomplete repair in the preparations, we undertook the detailed study of the structure of irradiated and control disks.

The architecture of the imaginal disk of the wing was reconstructed with the aid of sketches of sections which followed each other in a series. For this research we selected disks from Experiments X, XI, and XII (irradiation of 4-day larvae with a dose of 2000 roentgens, fixation in a day) from larvae which were ready for pupation and control disks from larvae of the same age.

In studying the architecture of imaginal disks, one's attention is attracted to its uniformity, its standardization. The uniformity of their structure, particularly in the region of the "pinna," that is, the rudiments of the wing, turned out to be even greater than we had expected from the data obtained from the study of the overall preparations. An example of a typical control disk is shown in Figure 4. We note two very characteristic figures which are always observed in the control disk. 1) An extended ring inside the disk in the anterior part of the rudiments [beginning] of the wing, corresponding to the deepest-lying fold. Quite rarely, in 8-10 per cent of the disks, this fold had not yet formed a cavity and looked like a massive oval body that filled almost the entire disk. 2) A cross section through the middle of the "pinna," which looks at this stage like a wide, low cup with a regular, almost flat, bottom (this last figure is presented in Figure 2a).

A different picture is observed when one examines the imaginal disks of irradiated larvae. Many disks do not differ noticeably from the control disks in respect to their structure. The most frequent change involves not the shape, but the size of the disk. Examples are encountered which are decidedly smaller or of intermediate size. It is notable that even dwarfed disks can have a wholly normal form. Quite frequently, however, one may observe more or less far-reaching simplifications in the architecture of the small disks. The most noticeable of these is the lack of the figure of the ring (Figure 5). Disks of normal or almost normal size most frequently have a normal structure. However, even among them one may encounter examples of simplified structure. The figure of a ring may be entirely lacking or the ring may be represented by a small oval body lying in its place which is one-half-one-third the size of the figure of a cup which follows. More rarely one discovers changes which can most readily be described as distortions of the normal structure. The most characteristic figures of the rudiments of the wing are distorted. The "ring" may turn out to be destroyed in

part of the sections, or instead of a flat bottom, the "cup" may have a bottom that has a complicated, curved, evidently abnormal structure (Figure 6).

The changes enumerated above are the most clear-cut deviations from the norm. Recording these changes is not difficult and permits one to make some interesting comparisons. In Experiment X 23.6 per cent of the flies had notches in their wings and the rudiments of the wing were sharply changed in 20 per cent of the studied disks. In Experiment XI KN mutations were found in 14.9 per cent of the imagoes and sharp changes in the architecture were recorded for 12 per cent of the disks. For Experiment XII the corresponding figures are 10.5 and 9 per cent. Thus, we have not only an undoubted parallelism between these two series of figures, the higher the percentage of mutants the more frequent sharp changes in the rudiments of the wing, but also a good correspondence of both indices in each of the three experiments. One may consider it probable, however, that the discovered sharp changes in the architecture of the wing rudiments constitute a morphological expression of incomplete repair, the ultimate consequence of which is mutation in the adult individual.

Discussion

X-rays and other types of ionizing radiation exert an especially destructive effect on undifferentiated cells (Zavarzin and others, 1936; Olenov and Pushnitsyn, 1952; Russel, 1954). The death of part of these cells in the rudiments [beginnings] of definite organs causes more or less sharp deviations in their further development. As a result there occur changes in the structure of adult individuals which had been irradiated in early stages of development.

Roentgenization causes the destruction of part of the cells of the rudiments of a definite organ in all or in the overwhelming majority of larvae. Consequently, a normal wing in an individual irradiated during the larval stage is always or almost always the result of repair. However, it would be a mistake to think that deviations from the norm, or mutations in adults, such as the "notched wings" we studied, are the direct consequence of the annihilation of part of the cells, a structural defect caused by the lack of these cells. The thing is far more complicated than that.

Lees (1941) showed that even in white pupae mechanical destruction of part of the wing rudiments never leads to formation of the corresponding defect (small holes) in the lamellae of the wing. Only when the wing is already completely formed (not far from emergence) does repair of the defect fail to follow its destruction. On the other hand, in this late stage, as our experiments have shown, roentgenization no longer causes the appearance of KN mutations. Thus, this mutation, like

others, is not a simple structural defect brought about by lack of part of the cells. Irradiation of the developing organism not only causes some of its cells to die, but may destroy the normal course of morphogenetic processes as a result of incomplete repair [See note].

([Note] If this conclusion had to be justified for other cases, as it was in respect to the KN mutation, for example, the appearance of supplementary veins on the wings as a result of mechanical trauma [Henke, 1933] or roentgenization [in our experiments], it [the conclusion] is wholly obvious. The transformation of part of the rudiments of the eye into a foot-shaped growth [Waddington, 1942 a and b; our experiments] is likewise, as in all heteromorphoses, the result of incomplete repair.)

What then, is the connection between the death of part of the cells and the ultimate result, the deviation from the norm on the part of the adult individual? Waddington (1942 a, b) suggested that when young larvae are irradiated determination is still labile, thus full regulation is possible, but that later this full regulation is not feasible. This explanation cannot be accepted since in older larvae, white pupae, and even in the beginning of the pupa stage there is no strict determination of the cells of the wing buds [rudiments]--the completion of determination means the limitation of potentialities; but actually this is not observed (Lees, 1941). We believe that the result depends not upon the degree of determination at the time of irradiation, but on which period of development of the organ bears the whole weight of the repair process. If the repair process is completed before the period characterized chiefly by multiplication of the cells of the imaginal disk is ended and before the morphogenetic dependences which determine the further development of the wing rudiments come into play, then a complete return to the norm is readily feasible. If, on the other hand, the process of repair is going on at the same time that the structure of the organ is being determined, then the intersection of these processes can lead to far-reaching changes in the definite structure. This critical period, the period of determination of many of the most important peculiarities of structure of the imaginal organs includes, as is well known, in Drosophila the final hours before pupation and the white pupa stage (Waddington, 1939, 1940, and others).

Here it seems timely to pose the following question: how to connect the data from Geigi [1931] and Howland and Child [1935] on defects of imaginal organs observed in Drosophila following influences exerted on them in the embryonic stage of development with the possibility of full regulation of injuries inflicted far later. The question inevitably arises as to whether even in the embryonic stage the "regenerative territory" consists of a very few cells, destruction of which would lead to irreparable defects.)

The explanation now offered in regard to the dependence of the effectiveness of external influences on the stage of development at which these influences are exerted reveals the actual sense of the widely used term "sensitive period." This term is usually applied to designate

the stage of development in which the external influence has the greatest effect, assuming that the readiness with which changes are obtained provides evicence of the special sensitivity of the cells and tissues at the given stage. When the question is posed in this way, much remains not understood, in particular the noncoincidence of sensitive periods in respect to different external influences (rays, temperature effects, chemical factors) which was repeatedly noted, for example, by Epsteins (1939). Bearing in mind the meaning of the repair process, we become able to determine correctly the meaning of sensitive periods.

The repair of injuries inflicted by various factors proceeds at different rates; it is particularly protracted in the case of damage from radiation agents (Aleksandrov, 1952). Therefore, when the influence is exerted at the very same stage of development in some cases the repair process will destroy the determination under way, in others it will not. In general, one may say that the sensitive period for a given indication, or for a given external influence, is often that stage for which the process of repair (which takes place after injury is inflicted) coincides with the period in which the indication is determined [see note].

([Note] Of course, the vulnerability to injury of cells can be different for different stages of ontogensis and stages of histogenesis. The usual interpretation of the concept of "sensitive period" is based on these differences. But, as has been pointed out, the final result depends not only on the state of the cells at the time the influence was exerted, but also on subsequent events. Thus, the explanation offered here includes in itself as one of the links, a characterization of the state of the cells subjected to the influence.)

In what way, then, does the death of part of the cells of the imaginal disk brought about by radiation and the subsequent process of repair lead to the occurrence of the KN mutation? A change in the conditions under which the development of an organ takes place is a result of the death of part of the cells. As noted by Waddington (1942 b) and also by Villee (1946) the multiplication of cells, the growth of the imaginal disk, the turning of its layers turn out to be variable as a result of the elimination of a part of the cells and the accumulation of the products of their decomposition which may perhaps be of morphogenetic meaning. On the other hand, it is possible that some dead cells still had been destined to produce substances which play the role of inductors and there is a deficit of these substances. Finally, the role of cellular detritus as a mechanical obstacle for the normal development of the imaginal disk is not excluded. In the final analysis, even though the resorption of decomposition products proceeds successfully in all individuals without exception, repair does not always turn out to be complete.

Studies of the architecture of the imaginal disks of larvae prior to pupation revealed that the structure of the rudiments of the wings in part of the irradiated individuals differs from the control--it is either simplified or distorted.

When evaluating the significance of this fact, it is essential to bear in mind that the mutation which causes notches in wings reveals its action even in white pupae, bringing about changes in the distribution of the remaining cellular material in respect to the veins outlined in this stage (Waddington, 1939). In the preceding stage, in larvae prior to pupation, we found changes in the architecture of the rudiments of the wings of irradiated individuals, and these changes were encountered in each experiment with about the same frequency as the "notched wings" mutation appeared in adult individuals. Thus, we have sufficient grounds for assuming that the changes in the architecture of the imaginal disks constituted the first indications of future mutation. These changes in the structure of the imaginal disks provided evidence that the normal course of morphogenetic processes was destroyed in the process of repair of the imaginal disks, which is also the reason for the changes in the structure of definite organs--the x-ray mutation.

Conclusions

1. Roentgenization of larvae and pupae causes the destruction of part of the cells of the imaginal disks--the rudiments of definite organs of the insect. X-ray mutations are the result of the incomplete repair of this injury. Their frequency depends upon the time of repair.

2. The data obtained permit one to give a new interpretation of the concept of the "sensitive period" in ontogenesis. The stage for which the repair process caused by the inflicted injury coincides with the period in which the indication is determined is frequently the sensitive period for a given indication, for a given external influence.

3. The destruction of part of the undifferentiated cells and the deviations caused by it in the further course of ontogenesis are obviously a general characterization of the effect of ionizing radiation on the developing organism. One should have this picture in mind when working out a theory of the biological action of ionizing radiation.

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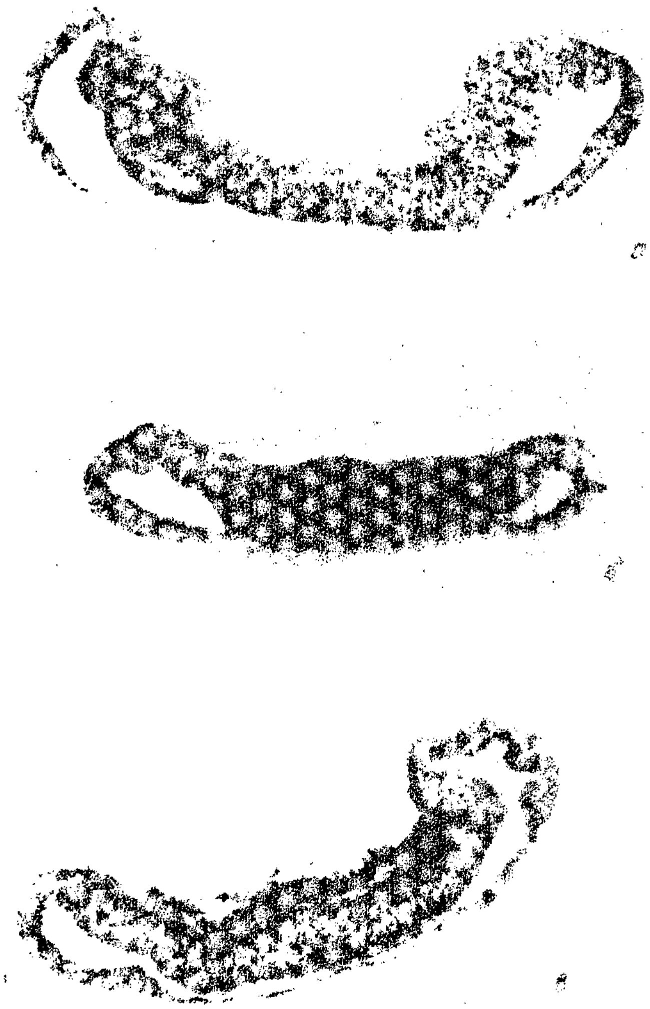
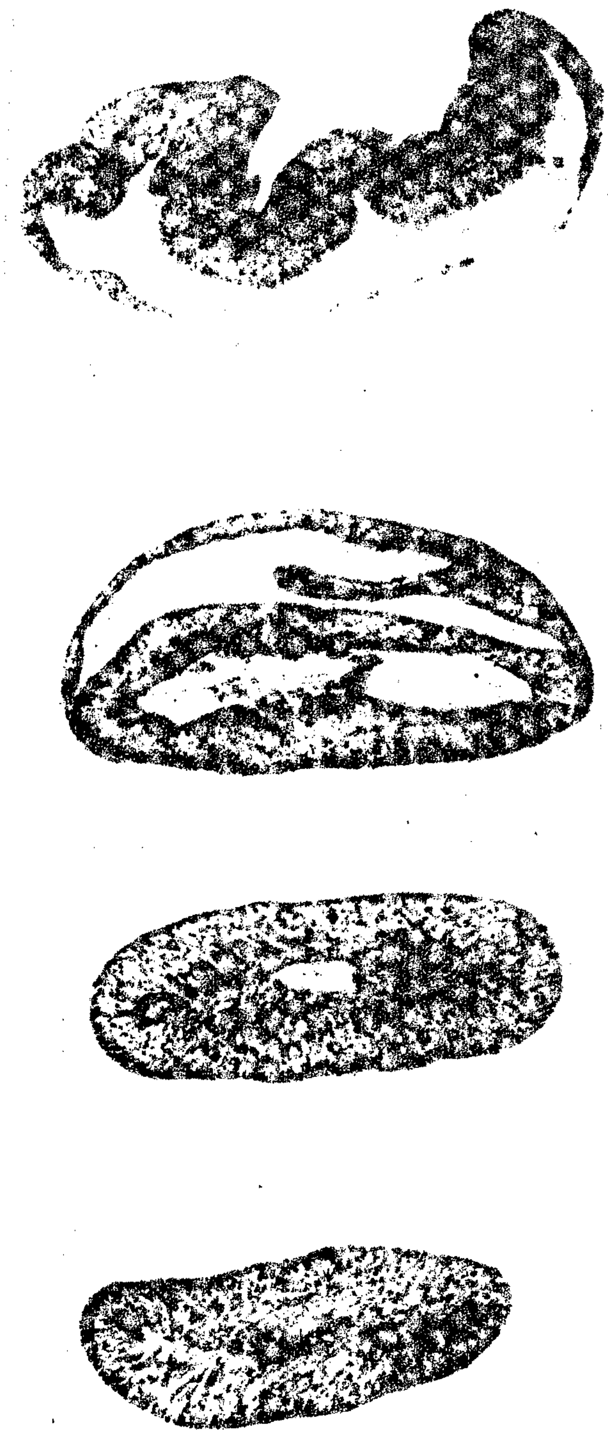
FIGURE APPENDIX

Figure 1. KN morphosis. Normal wing to the right.

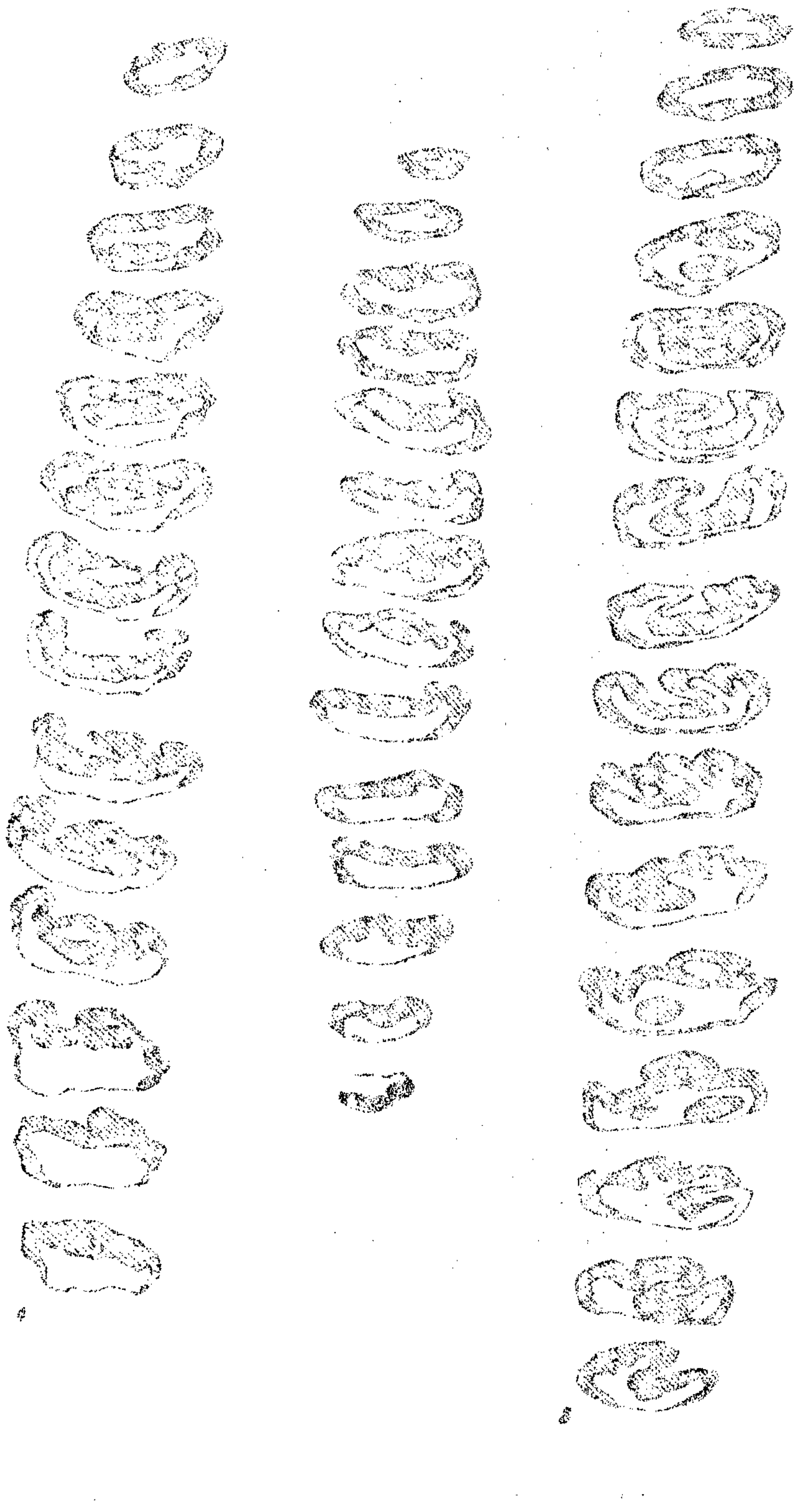


a
b
c

Figure 2. Imaginal disks of larvae.
a - control; b - irradiated, 12 hours after irradiation; c - irradiated, one day after irradiation.



ure 3. Imaginal disks of pupae. a - white pupa (irradiated in larval stage); the same, with decomposition products inside the disk; c - control pupa, after ghtening of folds; d - pupa, irradiated in the larval stage, after straightening lds.



4 (5) 6

s 4-6. 4 - series of sections of a disk from a control larva. 5 - series of
ns of a disk from an irradiated larva. Absence of "ring." 6 - series of
ns of a disk from an irradiated larva. Curvature of "cup."

REVERSIBILITY OF DIFFERENT FORMS OF RADIATION INJURY IN DIPLOID YEAST CELLS

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Gamma-ray irradiation of diploid yeast cells that are in a state of mitotic dormancy causes the death of such cells while "under the rays" or soon after irradiation (first form of injury), the loss of reproductive capacity by the irradiated cells after one, rarely two gemmations (second form of injury) or after four cycles of multiplication (third form of injury), it also reduces the rate of formation of macrocolonies with cells not inactivated by irradiation (Korogodin, 1957 a, 1958 a). If, on the other hand, yeast cells that have been irradiated with partially lethal doses are incubated prior to sowing in nutrient agar in sterile tap water, the degree of injury of the population is decreased. At the same time the percentage of survival increases steadily, as the duration of such postradiational incubation increases (Korogodin, 1958 b).

Two questions arise with the foregoing statements.

1) Is it true that the phenomenon observed in irradiated yeast cells of increased survival due to postradiational mitotic dormancy is an effect of restoration?

The fact is that in the work cited above (Korogodin, 1958 b), the survival percentage of irradiated yeast cells S is determined by the microcolony method, that is, by means of the equation

$$S = \frac{s}{s + m} 100\%$$

where s is the number of normal colonies counted in the preparation (we shall denote them by the index IV) and m is the number of pathological forms (I + II + III). The term $\frac{s}{s+m}$ may increase as a consequence of true restoration (that is, the transition of cells from class m to class s) as well as through lysis of the more severely injured cells (decrease in class m), partial multiplication of not inactivated individuals in a nonnutrient medium (increase in class s) or a combination of both the latter possibilities. We note incidentally that Sherman and Chase (1949) suggested the last variant to explain the increase described by them in the number of macrocolonies in Petri dishes when irradiated yeast was kept in a nonnutrient medium.

In the preceding work (Korogodin, 1958 b) the conclusion in respect to the existence of a restoration effect was drawn on the grounds that when yeast cells irradiated by partially lethal doses were incubated in nonnutrient agar (water + 2% agar), they did not differ from the control

in the rate of spontaneous lysis, and gemmation induced by irradiation was not observed. However, whether these laws were preserved when irradiated cells were incubated in a liquid nonnutrient medium remained unexplained. The first part of the research work, the results of which are set forth below, was devoted to a study of this question.

2) The second question, which made sense only after final proof of the existence of the restoration effect, amounts to explaining the connection between this phenomenon with some form of reaction of yeast cells to gamma-ray irradiation. In other words, it was necessary to study the possibilities of restoration in the second and the third [types II and III] types of injury and retardation of the rate of formation of macrocolonies. The first form [type I] of injury, that is, inactivation without preceding gemmation, as was shown previously (Korogodin, 1958 b), is irreversible (at least under the conditions of our experiments).

This work was done in the summer of 1958 at the Miassovo Biological Station. The basic part of the mathematical processing of the data obtained was carried out by N. V. Luchnik. The authors take advantage of this opportunity to express their sincere gratitude to N. V. Timofeyev-Resovskiy and N. V. Luchnik for their constant attention and their assistance in judging the results, also to the entire staff of the Laboratory of Biophysics of the Institute of Biology of the Urals Branch, AN, USSR for their daily cooperation and conduct of research work.

Object and Methodology

Diploid yeast Saccharomyces vini, of the Megri-139-V strain served as the object of study. A water suspension of the cells (concentration 6-7 millions/milliliter) in a 72-hour must-agar culture, containing not more than 0.1-0.3 per cent of gemmating individuals was subjected to irradiation. Co^{60} was used as a source of gamma rays. In all experiments the dose amounted to 30,000 roentgens at a power of 670 roentgens/minute. When the yeast was sown on a nutrient medium immediately after irradiation, such a dose caused inactivation of 24.6-1.8 per cent of the cells after one-two cycles of multiplication (type II injury) and 42.8-3.2 per cent of the cells in the stage of multicellular microcolonies (type III injury). The mean survival for microcolonies amounted to 32.8-2.4 per cent and for macrocolonies 35.7-4.2 per cent.

The irradiated and the control suspensions were incubated at 30 degrees in sterile lake water. Immediately after irradiation and later at definite intervals of time, samples were taken to determine the concentration of cells (counting in a Goryaev chamber), the percentage of gemmation (by microscope, by checking 1500-2500 cells), and the

degree of damage of the population--by the standard method of macrocolonies (the number of colonies in Petri dishes were counted after 48, 72, and 96-hour incubations at 30 degrees) and by the method of microcolonies, which permitted determining the ratio of the different forms of injury. The experiments were repeated 3-5 times.

The effect of postradiation incubation on the different forms of radiation injury in yeasts was studied in the following way. After irradiated yeast cells were kept for varying periods of time in a nonnutrient medium, they were sown on glass slides covered with agarized must (must 5^{o} Ball. - 2% agar) and incubated at 30 degrees. Every 3-4 hours of maintenance in a thermostat, for the duration of a 36-hour incubation, the preparations were studied under a microscope. At the same time, the sections of the preparations were controlled in such a manner that there would be 150-300 developing microcolonies under observation in each preparation. Taking into account the ratio of the forms of the microcolonies during the entire incubation provides information on the dynamics of the development of the colonies made up of cells damaged by radiation to various degrees and also makes it possible to avoid errors due to partial lysis of early inactivated cells, encroachment on pathological colonies by actively mutliplying neighbors, or merging of the descendents of several adjacent cells into one colony. Parallel evaluation of survival percentages by macrocolonies and microcolonies showed that when yeast was grown on a solid nutrient medium for 24 hours, not all the cells capable of prolonged mutliplication succeeded in forming disk-shaped microcolonies of the proper form. Such evaluation also permitted making suitable corrections in the results of counting the relative content in the preparations of cells which were inactive after several cycles of multiplication.

Table 1

CONCENTRATION, GEMMATION, AND VIABILITY OF IRRADIATED AND NOT-IRRADIATED YEAST CELLS WHEN THEY WERE INCUBATED FOR 24 HOURS IN A NONNUTRIENT MEDIUM

Duration of Incubation (in Hours)		0	2	4	6	12	19	24	M±
Concentration (in relative units)	Experimental	100	96	95	98	103	105	102	99.6±1.3
	Control	100	101	105	107	100	96	98	101.4±1.7
Gemmation (in per cent)	Experimental	0.30	0.43	0.50	0.40	0.71	0.31	-	0.44±0.05
	Control	0.20	0.49	0.54	0.70	0.30	0.40	-	0.45±0.08
Survival percentage of the irradiated cells (in per cent)	Macrocolonies	29.3	-	39.6	-	66.2	-	73.6	-
	Microcolonies	30.7	-	34.0	-	58.0	-	67.5	-
Content of viable cells in the control (average per Petri dish)		131±1.2	-	115±2.8	-	126±2.5	-	121±5.7	126±2.9

Results

Lysis and gemmation in a nonnutrient medium. The results obtained from studying lysis and gemmation in irradiated and not irradiated yeast cells are presented in Table 1. In view of the good reproducibility of the results, data from each series of repetitions of the separate experiments were used to compile the table.

The results presented in the table obtained from determining the concentrations of yeast cells in the suspensions and the relative content of gemmating forms provide evidence of the stability of these indices maintained in the control and the irradiated groups at the same level throughout the entire incubation. Their variability lies within the limits of error of the experiments. It is obvious that keeping yeast cells in the nonnutrient medium, irrespective of their preliminary irradiation, was not accompanied under the conditions of our experiments by either significant lysis or by material new growth or budding.

This conclusion is well confirmed by the results of studying the viability of the control and the irradiated yeast cells by methods of macrocolonies and microcolonies. In fact, the relative content of viable cells in the control is maintained at the same level throughout the entire period under study, while the content of viable cells in the irradiated suspensions increased noticeably in the same period. Registration of the increase in the number of viable cells by both methods yielded results which coincided well, thereby proving that the relative increase in the content of normal forms of microcolonies correctly reflected the absolute increase in the quantity of viable cells in the irradiated suspensions during their postradiational incubation in a nonnutrient medium.

Thus, when irradiated yeast is incubated in a nonnutrient medium, there is neither selective lysis of irreversibly damaged cells, nor multiplication of the cells which have retained their capacity for reproduction, but the registered effect of an increase in the quantity of cells which are capable of prolonged multiplication is the result of their true restoration from radiation injury.

Dynamics of the development of microcolonies. Now let us examine the data on the effect of postradiational incubation of yeast cells in a nonnutrient medium on the rate of formation of microcolonies and on the ratio of different forms of injury.

In Figure 1a curves are presented showing the changes in the ratio of different forms of microcolonies depending upon the duration of their growth in a solid nutrient medium when sown immediately after irradiation and 24 hours after irradiation. Curves I show the decrease with time of single cells which form buds and are transformed into microcolonies of 2, then of 3 or 4 cells; curves II show the increase, then the decrease of microcolonies of form II; curves III show analogous changes in the content of microcolonies of form III in the preparations:

curves IV show the same thing for normal disk-shaped microcolonies. As noted above, yeast cells irradiated with a dose of 30,000 roentgens experience inactivation either at the stage of the 2-cell - 4-cell microcolony, or at the stage of "chains" or "branches." The position of right horizontal segments on the y-axis of curves II, III, and IV depicts a final picture of the content of two forms of injury and normally viable cells in the preparations. It may be seen from the figure that stabilization of the relative content (in the preparations) of microcolonies of type II, as distinguished from other types of microcolonies, occurs even after 10-13 hours when they are cultivated at 30 degrees.

When corresponding curves of Figure 1 are compared, one may readily see the acceleration of the formation of microcolonies by irradiated cells kept in a state of mitotic dormancy prior to sowing as compared to cells which were sown immediately after irradiation. To analyze the changes in the rate of formation of microcolonies of different shapes, it is convenient to make the following transformation of the curves given in Figure 1a. Since these curves are the resultants of an increase in the corresponding type of microcolonies at the expense of a transition from the preceding type and a decrease at the expense of their transition to the succeeding type, then to determine the actual change in the relative quantity of microcolonies of each type it is essential, for each time point, to add the percentage of colonies of the type which interests us to the percentage of colonies of succeeding types. In this case one obtains for types II, III, and IV of colonies a series of S-shaped curves with different asymptotes. To compare the forms of these curves, they can be brought to 100 per cent (Figure 1b) and transformed into straight lines, applying the transformation of the logarithm of time--the PROBIT effect.

In Figure 1d are presented curves transformed in this way to convert type II microcolonies to type III and type III to type IV for both variations of the experiments. The corresponding transformed curves run parallel, that is, their form is the same, but the rate of the processes is different. The effective time (the time in which 50 per cent of the objects under study have undergone change -- ET50) for the conversion of type II microcolonies to type III when sown immediately after irradiation was 15.7 hours, and when sown 24 hours after irradiation 11.7 hours. Conversion of type III microcolonies to type IV required 28.0 and 20.3 hours respectively. The ratios of these numbers give the changes in the rates of conversion of some microcolonies into others under the influence of postradiational incubation. In the first case the ratio is 15.7 : 11.7 or 1.34 and in the second 28.0 : 20.3 or 1.38, that is, exceedingly close quantities.

Thus, postradiational maintenance of yeast cells in a nonnutrient medium makes possible a more rapid (in comparison with sowing directly after irradiation) formation of type III and type IV colonies. This acceleration is almost the same for both forms of microcolonies and averages 1.36. To check this conclusion, one may construct the curves

of both variants of the experiments, presented in Figure 1b, jointly, extending the time axis for the second variant (24 hours of postradiational incubation in a nonnutrient medium) by a factor of 1.36, as shown in Figure 1c. A very good coincidence of the corresponding curves of both variants of the experiments confirms the conclusion previously drawn that the effect of postradiational incubation on the rate of formation is the same for type III and type IV microcolonies, while the coincidence of the curves for type II microcolonies shows that the rate of their formation, that is, the rapidity of formation of buds by the individual cells sown on agar, undergoes precisely the same changes.

Figure 2 shows that the relative content of the different forms of microcolonies in the preparations incubated up to the final stabilization of these quantities (30-36 hours) depends upon the duration of maintenance of the irradiated cells in the nonnutrient medium. The curves are constructed on the basis of data from several experiments. For type II microcolonies, the content of which varied considerably in the preparations, the reliability of the restoration effect was confirmed by the results of regressive analysis ($P=0.029$). The numerical data presented in Table 2 show that the increase in the quantity of viable cells observed during the 24-hour postradiational incubation took place at the expense of a roughly equal decrease in the relative number of cells which died both after 1-2 gemmations as well as after several cycles of multiplication.

Table 2

Final Ratios of Different Types of Microcolonies When Cells Were Sown on Nutrient Agar Immediately after and 24 Hours after Irradiation (in Per Cent).

Types of Microcolonies	Sown Immediately after Irradiation	Sown 24 Hours after Irradiation	N_{24}/N_0
I	0	0	-
II	24.6±1.8	12.0±0.8	0.49
III	42.8±3.2	23.0±2.8	0.54
IV	32.8±2.4	65.0±4.8	1.98

Thus, postradiation incubation of yeast cells which have been irradiated with gamma-rays in a nonnutrient medium markedly decreased the delay, caused by irradiation, of the first gemmation of these cells and formation by them of different types of microcolonies when sown in a nutrient medium. At the same time, we observed a marked reduction in the lethal effect of irradiation due to a decrease in the quantity of cells inactivated after one or several cycles of multiplication.

The rate of formation of macrocolonies. The concluding part of the research was devoted to a study of the effect of postradiational incubation of yeast cells in a nonnutrient medium on the rate at which they formed macrocolonies, which, as was shown previously (Korogodin, 1957 a) decreases proportionally with the dose of radiation within a range of 12 to 72 kiloroentgens.

In Figure 3 we have shown the dependence of the survival percentage (by macrocolonies) of irradiated cells upon the duration of their postradiational incubation in a nonnutrient medium. Curve S_1 was obtained by counting the macrocolonies after they had been growing for 48 hours at 30 degrees and curve S_2 after 96 hours of growth, that is, after a period in which all viable cells succeeded in forming macrocolonies. The ratio S_1/S_2, that is, the relative rate of formation of macrocolonies, by irradiated cells is shown by curve E.

It follows from figure 3 that the increase in survival percentage of the cells due to their postradiational incubation in a nonnutrient medium is accompanied by an increase in their rate of formation of macrocolonies. At the same time, both quantities change in an almost parallel manner over a range of 4 to 24 hours of incubation in a nonnutrient medium. Consequently, the effect of restoration is extended both to the lethal action of readiation as well as to the retardation induced by irradiation of the formation of macrocolonies by yeast cells not inactivated by gamma-rays. It also may be seen from Figure 3 that under the conditions of our experiments the processes of restoration were almost wholly completed in the course of 24 hours of incubation of the irradiated cells in a nonnutrient medium.

Discussion

The data presented above provide final confirmation of the presence of the effect of restoration in diploid yeast cells kept in a state of mitotic dormancy after irradiation with gamma-rays. It was shown that this restoration was extended to both types of postradiational inactivation of the cells and to the delay in their multiplication induced by irradiation and affecting the first and subsequent cycles of gemmation.

The delay in the first gemmation in yeast cells irradiated by gamma-rays is, apparently, a consequence of injury to all the cells as a whole (the protoplasm and the nucleus). This is borne out by the regularity of distribution of this delay among the cells of the irradiated population (Korogodin, 1957 b) and the changes in the physioochemical properties of the cell correlated with this delay which appear, with moderate doses of radiation, in intensified brightness of luminescence of such cells after they are stained with acridin orange (Birukov and others, 1958).

Chromosome abberations are apparently the cause of postradiational death of diploid yeast cells, as many authors believe (Nybom, 1953; Zircle and Tobias, 1953; Tobias and others, 1958; and others).

Delayed formation of macrocolonies by irradiated yeast cells, as the data obtained by Tobias and others (1958) permit one to assume is the consequence of two causes, namely: an increase in the duration of several of the first cycles of gemmation in irradiated cells and a partial dying away of their descendents in 4-5 generations. The partial death of the descendents of irradiated diploid yeast cells is apparently very unevenly distributed through the population capable of forming macrocolonies of individuals (refer, for example, to the variation curves of the sizes of macrocolonies in Korogodin's work, 1957 a) and may be caused by delayed pathological changes in the nuclear apparatus of such cells. It is very probable that the duality of the reason for delay in the formation of macrocolonies by irradiated yeast cells is also expressed in differences in the forms of curves S_2 and E (Figure 3) in early intervals of postradiational incubation.

Thus, the full picture of postradiational changes in mitotic activity of diploid yeast cells is a consequence of both cumulative as well as localized action of ionizing radiation on cellular structures.

The phenomenon of postradiational restoration in the "cumulative" effect of irradiation, namely, in the retardation of the first mitosis in sea urchin eggs irradiated by x-rays was described by Henshaw and others, and analyzed by Lea (1955). Postradiational restoration was also described for the cells of a culture of tissues of chick embryos (Strangeways and Fell, 1927), the mechanism of radiation injury in which, apparently, has a mixed character, and for the bacteria Escherichia coli (Pratt and others, 1955, and others), the problem of the mechanism of radiation injury of which is still under discussion (Shekhtman and others, 1958), but no analysis has been made in these works of the effect of mitotic dormancy on the consequences of cumulative and local influences of irradiation. Simultaneously with our work and independent of us, Luchnik and Tsarapkin (1959) discovered a restoration effect, analogous to the one we described for diploid yeast cells, for the chromosomes in cells of damaged pea roots. However, the data on the character of postradiational restoration of different types of radiation injury in the same object, as far as we know, was obtained here for the first time.

The reactivating effect of postradiational mitotic dormancy on the growth of tissues in culture, on the delay of fission of sea urchin eggs, on the inactivation of intestinal bacilli, on the amount of chromosome rearrangement in pea root cells, and on different types of postradiational changes in diploid yeast cells apparently indicates the similarity of the primary radiobiological processes which form the basis of the effects noted here. The fact that the destruction of mitotic activity serves as the index of injury is common to the cases of reactivation cited above. In order to determine this destruction it is necessary

to place the irradiated cells in conditions favorable for multiplication. The occurrence of mitosis is the event, which is, so to speak, "decisive," that reveals the unknown primary destruction in the cell. The later this event occurs, the less lethal the consequences of irradiation for the cell.

The data presented above once again confirm the ideas that the primary radiobiological injuries to cells are to a certain extent potential, capable of spontaneous disappearance, and acquire an irreversible or clear-cut form only after one or several mitotic cycles (Korogodin, 1958 b). It is very probable that the primary radiobiological injuries are composed of reversible and irreversible components, the relationship of which depends upon the linear density of ionization and the physicochemical conditions prevailing at the time of irradiation. These assumptions seem justified to us, at least for the influence of sharply ionizing radiation on the biological effects connected with injury to cytoplasm and destruction of chromosomes.

It is not clear yet whether the transition of potential, reversible radiobiological destruction to irreversible destruction after irradiation is completed during one mitotic cycle or whether it may be extended over several successive cycles of cell multiplication. One may assume, however, that the probability of realization of primary potential radiobiological destruction decreases with an increase in the interval of time between irradiation and the occurrence of mitosis, and depends upon the physiological state of the cells and the physicochemical conditions of the medium (refer, for example, to the work of Korogodin, Tarusov, and Tambiev, 1959). The physiological state of cells can be determined by the conditions of their care before and after irradiation and the effect of radiation on various cell components, injury to which would not necessarily lead to the death of the cells. Thus, under the influence of radiation, as N. V. Luchnik believes, a certain amount of toxic products might be formed in a cell which would affect mitosis, destroying or retarding the synthesis of substances essential for normal functioning of the cell.

It is difficult as yet to say anything in regard to the nature of primary (potential) radiobiological injuries. In the case of a lethal effect on diploid cells this may be actual breaking of the chromosomes, the probability of restitution of which is proportional to the duration of mitotic dormancy, or potential (hidden) injuries to the chromosome apparatus, the realization of which occurs at the time of mitosis and depends upon the cumulative effect of irradiation, or, finally, a chain of physicochemical reactions which are capable of leading to chromosome damage in dividing cells, but diminishing during a period of mitotic dormancy. One cannot exclude the possibility that the mechanisms of chromosome abberrations are manifold under the influence of ionizing radiations and that the primary processes which form the basis of postradiational restoration are then just as manifold.

Conclusions

1. This work presents proofs of the existence of the restoration effect in diploid yeast cells irradiated by gamma-rays when they are incubated in a nonnutrient medium after irradiation.

2. It has been shown that postradiational mitotic dormancy [rest] is accompanied by a decrease in the biological consequences of irradiation connected with cumulative effects (retardation of the first mitosis), local effects (two types of postradiational inactivation), and mixed effects (delay in the formation of macrocolonies) on the cells.

3. A working hypothesis is used to explain the effect of restoration. According to this hypothesis, the primary radiobiological injuries can have a potential form and a reversible character. The probability of their transformation into irreversible changes is in inverse proportion to the duration of postradiational mitotic dormancy and is connected with the physiological state of the cells and the physicochemical conditions of the medium.

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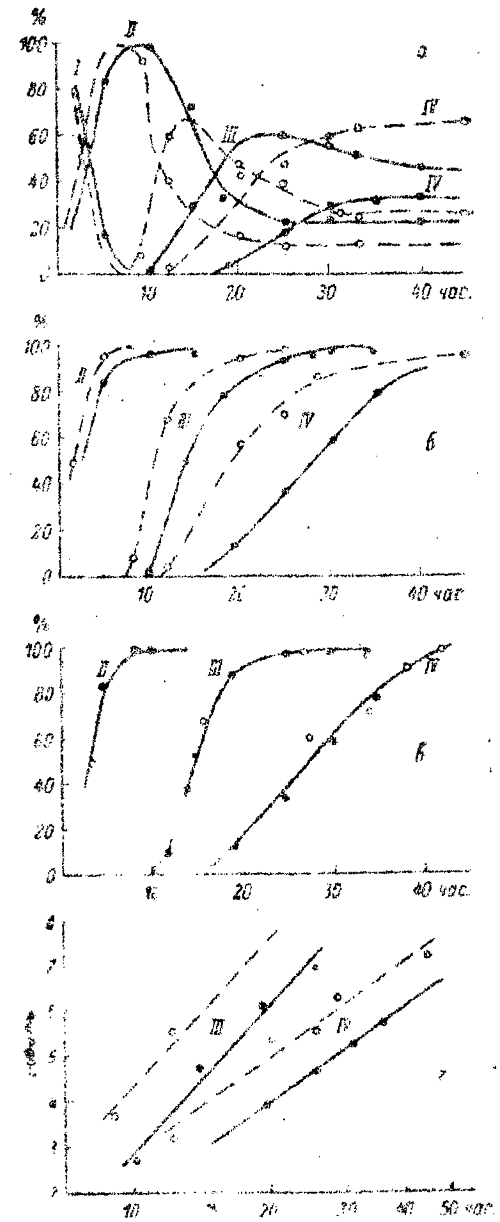
FIGURE APPENDIX



Per cent
100

0
10
a
40 hours

Per cent
100

0
10
b
40 hours

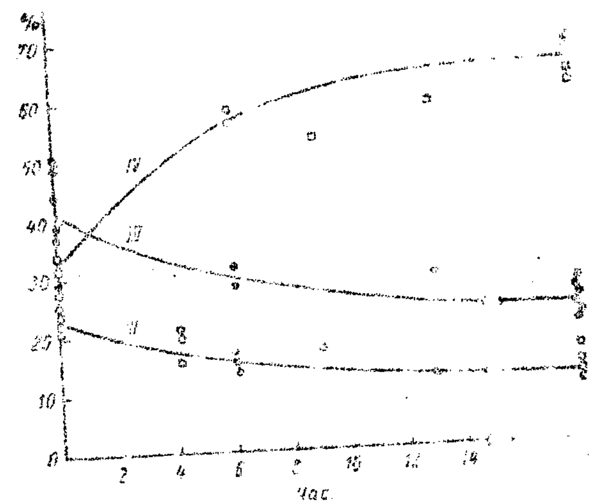
Per cent
100

0
10
c
40 hours

Per cent
100

0
10
d
40 hours

Figure 1. Dynamics of formation of microcolonies by irradiated yeast cells when sown in a nutrient medium immediately after irradiation (solid lines), and after 24 hours (dashed lines). Experimental data (a) and different methods for transforming them (b, c, d). Explanations in text. I, II, III, and IV - different forms of microcolonies.



Per cent
70

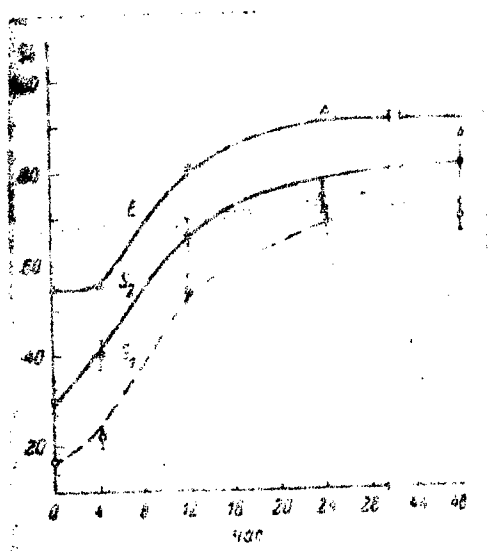
0

2

14

Hours

Figure 2. Final content Hours (in the preparations) of different forms of microcolonies (II, III, and IV) depending upon the duration of incubation in a nonnutrient medium.



Per cent
100

0 4 48

Hours

Figure 3. Relative content (in Petri dishes) of macrocolonies when counted after 48-hour and 96-hour incubation at 30 degrees and values of the index $E = S_1/S_2$ depending upon the duration of postradiational maintenance of irradiated cells in a nonnutrient medium.
S_1 - count after 48 hours; S_2 - count after 96 hours.

THE EFFECT OF GAMMA-RAYS ON MITOSIS IN A POLYPLOIDIC SERIES OF WHEAT

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As early as the end of the [nineteen] twenties it was shown that polyploidy is one of the cytogenetic factors which influences the biological effect of irradiation (Stadler, 1929). By the present time, a series of radiobiological experiments conducted on animals (Clark and Kelly, 1950), plants (Froeier, 1941), and microorganisms (Latarjet et Ephrussi, 1949) established that depression of growth and mortality in polyploidic forms usually turns out to be decreased. However, it was shown in the work of Froeier, Gelin, and Gustavsson (1941) that when ionizing radiations acted on mitosis, the polyploidic forms were the most sensitive. In view of this contradiction, it seemed to be of interest to check and to extend the results of these authors, all the more because the experiments they conducted were not irreproachable from the methodological standpoint, as they had fixed rootlets of definite length but at different times after irradiation in different variants of the experiments. However, it is known from the literature that the time of fixation has a material influence upon the results (Gaul, 1957).

A polyploidic series of wheat was selected for the experiments described below. Air dried seeds of the following species served as experimental material: Triticum monococcum (2n=14), Triticum dicoccum, Triticum durum V. Hordeiforme (2n=28), Triticum spelta, Triticum vulgar v. alborubum (2n=42), also the wheat-wheatgrass hybrid No (2n=42). The seeds were irradiated with a dose of 11,000 roentgens of gamma-rays from Co^{60} at the rate of 41.6 roentgens/minute. Immediately after irradiation the seeds were soaked in tap water for 12 hours, then germinated in wet sand in Petri dishes. The soaking and germination were carried out in a thermostat at a temperature of 24 degrees. 48 hours after the end of germination, the tips of the roots were fixed with Leviskiy's [fixing fluid] and stained with fuchsin-sulfurous acid (Feulgen's reaction) or with aceto-lacmoid (with preliminary Feulgen hydrolysis). The percentage of dividing cells was determined in temporary KVECH, preparations, and the anaphases were analyzed on the presence and character of chromosome aberrations; at this time fragments and chromosome bridges were taken into account.

The basic results of the experiments are presented in Table 1. It follows from the table that mitotic activity had been reduced quite sharply in all wheat specimens under the influence of irradiation. This reduction turned out to be approximately the same for all species

and does not depend upon the ploidy of the cells. As for the number of abnormal anaphases, this turned out to be widely varying for the different species. The percentage of abnormal anaphases increased with an increase in the ploidy. In wheat-wheatgrass hybrid No 1, however, the percentage of abnormal mitoses turned out to be higher than in wheat which had the same number of chromosomes.

A more detailed analysis of abnormal anaphases led to interesting results. The number of chromosome bridges and fragments in the cells was taken into account. It turned out that the degree of ploidy [haploid, diploid, triploid, polyploid] had a different effect upon these two types of changes. The number of bridges and the number of fragments per 100 anaphases is shown in Figure 1 and the same number per 100 chromosomes is shown in Figure 2. The results of statistical analysis of this material is presented in Table 2. If we start with the number of changes per 100 cells, then the number of bridges increases regularly with the degree of ploidy (the coefficient of regression of 15.2 ± 2.1 is statistically reliable); but the number of fragments showed practically no change (coefficient of regression 4.2 ± 6.8 is statistically unreliable).

Table 1

The Effect of Gamma-Rays on the MITOTIC Activity and the Percentage of Species of Wheat

	Control				Abnormal Anaphases in Different Species of Wheat				
	Number of Counted Cells	Number of Dividing Cells	Mitotic Activity (in %)	Number of Counted Cells	Number of Dividing Cells	Mitotic Activity (in %)	Number of Counted Anaphases	Number of Abnormal Anaphases	Number of Abnormal Anaphases (in %)
Triticum monococcum	3130	314	10.03	4005	211	5.26	197	57	28.93
Triticum dicoccum	6230	627	10.06	3058	214	6.99	150	67	44.60
Triticum durum	3000	324	10.80	3000	165	5.50	150	70	46.60
Triticum spelta	2640	288	10.89	3000	167	5.56	150	78	52.00
Triticum vulgare	3000	316	10.55	3000	191	5.95	150	83	55.50
Hybrid No 1	3097	282	9.11	3063	159	5.19	112	89	79.40
Total	21097	2151		19126	1107		909	444	
X^2			5.275			9.943			448
P			0.38			0.08			10^{-10}

Table 2

Results from Statistical Analysis of THE Dependence of the Number of Bridges and Fragments upon the Degree of Ploidy

Studied Dependence	Equation of the Straight Line	Coefficient of Regression	t(3)	P
Bridges per 100 anaphases	Y=2.9+15.2	15.2±--2.1	7.25	0.005
Fragments per 100 anaphases	Y=38.4--4.2	--4.2±6.8	0.63	0.59
Bridges per 100 chromosomes	Y=12.8--0.7	--0.7±8.2	0.087	0.94
Fragments per 100 chromosomes	Y=17.6--6.4	--6.9±0.2	43.8	0.0002

An entirely different picture is obtained if one counts the number of changes per 100 chromosomes. In this case the number of bridges did not change, while the number of fragments decreased regularly with an increase of ploidy. The analysis described above concerns only the five species of wheat; the observed pattern did not extend to the wheat-wheatgrass hybrid No 1.

The results obtained showed that the mitotic activity from the effect of gamma-rays did not depend upon the degree of ploidy, while the number of abnormal anaphases changed regularly. One may conclude from this that the effect of gamma-rays on the rate of cell division and on the occurrence of abnormal mitosis is not a single process. This corresponds to the results obtained by other workers in our laboratories (Timofeyev-Resovskiy, 1956; Luchnik, 1956). Therefore, the results obtained can be explained most simply as follows. The number of primary breaks per cell increased proportionally with an increase in the number of chromosomes. Moreover, an increase in the number of chromosomes increases the probability of reunion of fragments; thus an increase in the degree of ploidy means an increase in the relative number of bridges. This also explains the above noted difference in the relative change in the number of bridges and fragments per cell, or per 100 chromosomes, with an increase in the degree of ploidy. This fact does not extend to the wheat-wheatgrass hybrid No. 1. This fact forces one to direct special attention to study of the radiosensitivity of hybrids (whose kinship is remote or close), all the more so because the literature contains contradictory results which indicate the greater resistance to radiation of hybrids (Luchnik, 1957). According to the generally accepted theory of chromosome abberations (Lea, 1946), simple breaks in the chromosomes serve as the primary phenomenon. At the same time, the observed effect depends upon the number of breaks per cell and upon

the probability of union of the breaks. Analysis of our material is made more difficult by the fact that the bridges can appear both as a result of assymetrical translocation (based on two breaks) and as a consequence of the union of two sister chromatids (based on one break). In both cases an increase in the probability of union should mean an increase in the ratio of the number of bridges to the number of fragments. To explain this pattern, one must conduct experiments on a number of other polyploid series, also on hybrids of the same degree of ploidy. Such comparisons would make it possible to uncover the pattern of formation of chromosome bridges and fragments under the influence of irradiation.

I make use of this occasion to express my sincere gratitude for the assistance and advice given me by N. V. Timofeyev-Resovskiy and N. V. Luchnik.

Resume

To elucidate the effect of gamma-rays on mitosis in a polyploid series, the following species of wheat were taken: Triticum monococcum, Triticum dicoccum, Triticum durum, Triticum spelta, Triticum vulgare, and the wheat-wheatgrass hybrid No 1. Dry seeds were irradiated with a dose of 11,000 roentgens. The rootlets were fixed 48 hours after germination. The results of cytological analysis showed that the mitotic activity had declined about the same extent in all species of wheat, that is, it did not depend upon the degree of ploidy. The number of abnormal anaphases increased with an increase in ploidy. The quantity of chromosome bridges per 100 anaphases increased with increased ploidy, but the number of fragments remaind constant for the different species of wheat. One can conclude from this that the effect of gamma-rays on the rate of cell division and on the occurrence of abnormal mitosis is not a single process.

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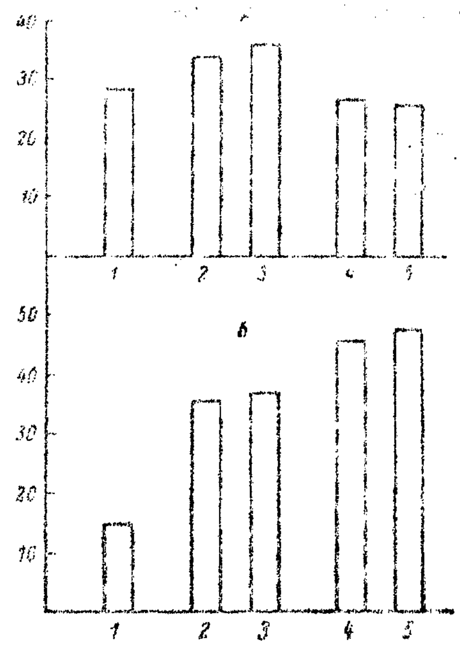
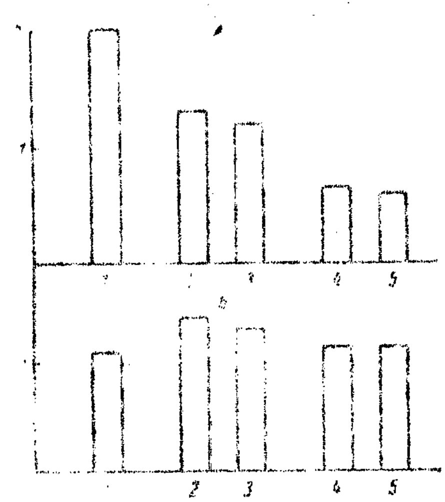
FIGURE APPENDIX





40 A

10

1 5

50 B

10

1 2 3 4 5

2

1 A

1 5

B

1

1 2 3 4 5

Figure 1. Quantity of fragments (A) and chromosome bridges (B) per 100 cells in different species of wheat after irradiation with a dose of 1,000 roentgens.
1 - Triticum monococcum (2n=14),
2 - Triticum dicoccum (2n=28),
3 - Triticum durum (2n=28),
4 - Triticum spelta (2n=42),
5 - Triticum vulgare (2n=42).

Figure 2. Quantity of fragments (A) and chromosome bridges (B) per 100 chromosomes in different species of wheat after irradiation with a dose of 11,000 roentgens.
1 - Triticum monococcum (2n=14),
2 - Triticum dicoccum (2n=28),
3 - Triticum durum (2n=28),
4 - Triticum spelta (2n=42),
5 - Triticum vulgare (2n=42).

RADIOSENSITIVITY OF THE MERISTEM OF BUDS AND RADICLES IN THE GERMS OF PEAS AND BARLEY

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When studying sensitivity to radiation we discovered a different reaction to irradiation of stems and roots in different plants. In peas and kidney beans the growth of shoots was most retarded, in wheat, barley, maize, the growth of roots was most affected. It was discovered in a number of studies that in the first mitoses of radicles there were more cells with chromosome rearrangements in radiosensitive species of plants than in species which were resistant to radiation (Gelin, 1956; Gelin, Ehrenberg, and Blixt, 1958; Khvostova and Valeva, 1959). The question arose: would a like parallelism be observed in radioresistant (growth retarded less) and in radiosensitive (greater retardation of growth) tissues of a single plant?

Table 1

Number of Cells with Chromosome REARRANGEMENTS in the Meristem of Radicles and Shoots in Pea Sprouts

Tissue	Dose (in Roentgens)	Number of Preparations Examined	Metaphases			Ana + Telophases				
			Total	With Fragments and Rings	Per Cent Injured	Total	With Fragments	With Bridges	Total Injured	Per Cent Injured
Meristem of shoots	15000	6	1014	173	17 $\pm$1.18	917	122	264	386	42$\pm$1.57
Meristem of radicles	15000	6	1702	137	8.1$\pm$0.21	1122	66	217	283	25$\pm$1.3

Table 2

Number of Cells with Chromosome REARRANGEMENTS in the Meristem of Radicles and Shoots in Barley Sprouts

Tissue	Dose (in Roentgens)	Number of Preparations Examined	Ana - Telophases: Total	Ana - Telophases: With Fragments and Bridges	Ana - Telophases: Per Cent Injured
Meristem of shoots	17000	20	2,679	857	32 ± 1.22
Meristem of radicles	17000	19	3,302	1684	51 ± 1.33

Seeds of Moscow 572 peas and Viner barley were taken for cytological analysis. The air-dried seeds were irradiated with gamma-rays from Co^{60} with an intensity of 600 roentgens per minute--the pea seeds with a dose of 15,000 roentgens and the barley with 17,000 roentgens. After irradiation the seeds were germinated in Petri dishes at 20-23 degrees. In the case of peas, the radicles and buds of the same plants were fixed by Atsetalkogol [apparently acetyle alcohol or methyl alcohol] 60-68 hours after soaking of the seeds. In the case of barley, we took radicles 3-5 millimeters long and apical buds with coleoptiles 5-7 millimeters long. This material was also fixed with acetyl alcohol. Damaged mitoses were counted in temporary acetocarmine preparations, counting the fragments in the metaphases, bridges and fragments in late anaphases and telophases. The results of cytological analysis were presented in Tables 1 and 2.

The tables indicated that in peas there was a markedly higher number of mitoses with chromosome rearrangements in the meristem of the shoots, while in barley this was true of the meristem of the radicles. Thus, the data obtained showed a positive correlation between the retardation of growth of the organ and the number of injured mitoses, which again provides evidence that the basic cause of retardation of growth of plants when the seeds have been subjected to ionizing radiations is injury to the cellular nuclei.

That retardation of the growth of stems and roots of peas depends upon the number of damaged mitoses is well illustrated in the figure.

It is well known that retardation of growth of plants after the seeds have been irradiated appears particularly clearly in the early stages of development of the plants. Further, plants grown from irradiated seed can attain the growth of the control plants due to multiplication of cells whose chromosome balance has not been destroyed.

Resume

It was discovered through cytological analysis of the tissues of the radicles and shoots in sprouts of peas and barley produced from seeds which had been irradiated with gamma-rays that there was a large percentage of cells with chromosome rearrangements in those tissues whose growth had been most retarded (the radicles in barley and the shoots in peas). Consequently, the radiosensitivity of tissues is linked with greater vulnerability of cellular nuclei.

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FIGURE APPENDIX

31.63 21.07 27.89 15.28

A B

K 15000 roentgens K 15000 roentgens

Inhibition of growth of radicles (A) and stems (B) and per cent of anaphases and telephases with chromosome rearrangements (black sectors) in the first mitoses after irradiation of air-dried peas with a dose of 15,000 roentgens of gamma-radiation.

The figures above the bars show the length of spouts in centimeters on the 28th day after soaking of the seeds. Germination temperature 20-23 degrees. In both cases the control is to the left and the result of the action of 15,000 roentgens is to the right. [The letter K to the left of the 15000 roentgens in the figure means control]

DISTRIBUTION OF THE PROTEIN TRYPTOPHANE IN THE TISSUES OF LARGE EMBRYOS AND IN CERTAIN ORGANS OF ADULT BIRDS

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According to the most widespread point of view, the basic significance of the amino acids which make up the molecule of protein consists in the creation of the specific organization of the protein molecule, its structure. In this connection, the role of the individual amino acids in the structure and the function of the protein molecule acquires particular interest. Tryptophane, as is well known, is one of the irreplaceable amino acids. Free tryptophane in the organisms of mammals serves as a source of nicotinic acid--vitamin PP. Biochemical studies cannot give a complete characterization of the protein composition of different organs since either total proteins or separate fractions of proteins extracted from ground organs are the chief objects of analysis. Histochemical methods permit one to reveal localization of individual amino acids contained in protein molecules, in some organ or another, in tissues, and even in separate cellular elements, and one may judge the relative content by the intensity of reaction (for example, staining of the preparation). Comparison of the data obtained from biochemistry and histochemistry permit one to give a more complete characterization of the proteins under study.

In previous works (Yushkevich and Kedrovskiy, 1956a, b, 1959; Kedrovskiy and Yushkevich, 1957), it was shown with the aid of the histochemical method that the organs and tissues of amphibians and mammals, which are similar in structure and functions, contain approximately identical amounts of protein tryptophane in the cellular plasm. Sharp differences in the tryptophane content in different organs within the limits of each class were established through histological differentiation of embryos. At early stages of development of the organs their component proteins contained small quantities of tryptophane which differed but little among themselves.

Histochemical data are set forth in this article on the distribution of tryptophane obtained from birds (for details of methodology, see Kedrovskiy and Trukhacheva, 1952; Yushkevich and Kedrovskiy, 1956 a). Special studies on the distribution of protein tryptophane in birds and their embryos have not, so far as we know, appeared in literature. In this connection, the objective of the research was to reveal differences in the distribution of protein tryptophane in the tissues of embryos and adult birds, also to compare birds with amphibians and mammals in this respect.

Certain organs and tissues of roosters (somatic muscles, heart muscle, duodenum, pancreas, liver, kidney, brain, spinal cord, skin, cartilage, lungs, testes, eyes) and large chick embryos from 6-day, 7-day, and 8-day incubation served as material for study. Fixing was accomplished with Shaffer's fluid and Carnoy's fluid with trichloroacetic acid (Yushkevich and Kedrovskiy, 1956 a); the material was poured into gelatin. The histochemical reaction was produced with the aid of Ehrlich's aldehyde (Kedrovskiy and Trukhacheva, 1952). Intensity of staining was determined at small magnification (eyepiece 7X, object 8X). Details were observed with the aid of large magnification (eyepiece 10 and 15X and object 40 and 90X). The head of the embryo was poured separately from the body. The body was separated into sagittal sections, the head was cut frontally, through both eyes and the large hemispheres. In order to compare the degree of intensity of the reaction in embryos and adults, the embryo (or its head) was poured in one block with a bit of tissue or an organ of the adult bird. In this way we were able to compare the reaction of embryonic and adult tissues in one section.

Results

The Organs and Tissues of Adult Birds

(In this work no attention was paid to irregular staining of nuclei.)

Blood vessels. The muscle walls of the vessels showed a reaction of moderate intensity. Erythrocytes were more lightly stained.

Muscles. The cross-striated muscle fibers (breast muscle) were clearly stained. The heart muscle was usually a bit lighter. The smooth muscle fibers (vessels, lungs, intestinal) reacted still less intensely.

Duodenum. The epithelium cells of the villi were stained with moderate intensity, but more lightly than the ring-shaped muscles. Sharp differences in the staining of the epithelium cells of the villi and the crypt which were characteristic of other classes (Yushkevich and Kedrovskiy, 1956 b; Kedrovskiy and Yushkevich, 1957) were usually not observed. Many smooth muscle fibers were stained in the stroma of the villi.

Pancreas. The end sections of the exocrine part of the gland were rich in tryptophane, which was localized chiefly in the granules of the secretion. The epithelium cells of the ducts reacted far more weakly. Tissue of the islands of Langerhans, which are found comparatively rarely in birds, were practically colorless.

Liver. The liver parenchyma reacted with moderate intensity. At times more brightly stained protein inclusions (Berg's lumps) were distinguished in the plasm of the liver cells.

Kidneys. The epithelium cells of the tubules of the kidneys were quite brightly stained, especially the apical parts of the cells and the Shchetochnaya Kaemka [Literally brush fringes]. The epithelium cells of the other tubules reacted more weakly. The glomerules were very lightly stained.

Large hemispheres of the brain. The general reaction was weak. The gray and the white substances differed little in tryptophane content. Some nerve cells were a bit outstanding from the general background in being stained.

Spinal cord. With generally very pale staining, the gray substance was colored a bit more brightly than the white. The motor neurons (chiefly the large ones) were stained more brightly than the basic substance of the glia. The erythrocytes in the vessels of the spinal cord turned out to be richer in tryptophane than the neurons.

Skin. The reaction of the epidermis was weakly positive. The sheaths of the down feathers reacted somewhat more strongly. The connective tissue layers of the skin were colorless.

Breast cartilage. The intermediate substance of the cartilage was stained slightly. The basic substance of the cellular territories was readily distinguished by its stain. Cartilage cells were scarcely stained.

Lungs. The lung parenchyma was very pale. The smooth muscle tissue which was strongly developed here reacted moderately intensely.

Testes. All tissue of the testes was stained to a pale color. Differences between the layers by the degree of reaction with Erlich's aldehyde, characteristic of mammals, could not be established here.

Eyes. The lens fibers were the most rich in tryptophane of all the tissues in birds. They showed an exceedingly intense reaction to tryptophane. The light-sensitive elements, the retinal layers, and the ganglionic layer of the retina were weakly stained. The remaining elements of the retina were practically colorless.

Embryos

Chicken embryos of 6, 7, and 8 days' development, when the formed rudiments of the majority of the basic organs would be distinguishable (Lillie, 1952; Shmidt, 1953), were taken for study. Chicken embryos of this age correspond roughly to 15-day rat embryos in respect to general development. Due to uneven development, some organs in these stages are closer to a definitive state than others. In other words, the embryos contain, along with slightly differentiated, more or less completely formed and functioning organs.

By studying the reaction of tissues from large embryos of all three terms of incubation (6, 7, and 8 days) with Erlich's aldehyde, we find a number of noticeable differences in the tryptophane content. After reacting with Erlich's aldehyde, sections of chicken embryos were stained a pale color. We were able to discover some differences in the reactions of different organs by using high magnification. The developing fibers of the back part of thr lens were distinguished by the highest tryptophane content. The intensity of this reaction could be compared with the reaction of the smooth muscles or the epithelium of the main sections of the kidney tubules of adult birds. As one went toward the anterior epithelium the color of the lens fibers became more pale. Accumulations of erythrocytes in the vessels were also quite brightly stained. The remaining organs and their rudiments were stained very lightly. Somewhat more brightly stained (in order of decreasing intensity) were: the white substance of the spinal cord, individual nerve tracts in the bodies of the embryos, ganglia, liver, heart, the developing rudiments of muscles, and the epithelial cells of some tubules of the mesonephros. On the eighth day the cellular layer about the bodies of future vertebrae and other parts of the skeleton were noticeably stained. The epithelium of the skin, intestine, the majority of the kidney tubules, the rudiments of the lungs and pancreas were stained very lightly. The cartilageous rudiments of the vertebrae and all the embryonic connective tissue showed practically no reaction to tryptophane.

Discussion

The tryptophane content differed in the organs of adult birds. Those which were rich in tryptophane: lens fibers, granules of the zymogen of the pancreas, somatic muscles, heart muscle; a moderate tryptophane content characterized: the liver, kidneys, smooth muscles, the epithelial cells of the mucuous membrane of the intestines; and the following were poor in tryptophane: the brain, erythrocytes, and testes.

A comparison of the results obtained for birds with those obtained for mammals and amphibians (refer to the table) showed that the majority of the organs of adult representatives of these three classes of vertebrate animals differed scarcely at all in respect to their protein tryptophane content. Organs which were similar in origin and functions were characterized by a like protein tryptophane content, both the organ as a whole, as well as its individual parts. The tryptophane content in the cytoplams of the tissues of embryos of representatives of the three classes under study was small at the stages studied and there were none of the sharp differences characteristic of mature tissues despite the differences noted above in the level of development of embryonic organs (the yolks of amphibian embryos were, of course, not taken into account). Organic differences in tryptophane content appearedin embryogenesis in the process of histological differentiation of the organs.

A more detailed discussion of results obtained for amphibians and mammals was carried in preceding reports. The results from the present study of birds confirmed the conclusions drawn previously. Apparently, the tryptophane content in the proteins of the majority of the organs and tissues studied is directly connected with their functions (Yushkevich and Kedrovskiy, 1956b, 1959; Kedrovskiy and Yushkevich, 1957; Kedrovskiy, 1958).

THE PROTEIN TRYPTOPHANE CONTENT IN THE ORGANS OF AMPHIBIANS, BIRDS, AND MAMMALS

Organs and Tissues		Amphibians (Axolotls, Frogs)	Birds (Roosters)	Mammals (Guinea Pigs, Rats)
Striated muscles		+++	+++	+++
Heart muscle		++	+++	+++
Smooth muscles		++	++	+++
Liver		++	++	+++
Kidneys	Main sections	+++	++	+++
	Other tubules	+,++	(+),+	+,++
Epithelial cells of the mucuous membrances of the duodenum		+++	++	+++
Pancreas	Exocrine lobules	+++	++	+++
	Granules of zymogen	++++	+++	++++
	Islands of Langerhans	(+)	(+)	(+)
		++	++	++
		+	-	++
		+	(+)	++
Skin	Epidermis	+	+	+
	Serous glands	+++	-	-
	Down cover	-	-	+++
Oocytes		++	-	++
Testes		(+),+	+	+
Peritoneal glands of the male		+++	-	-
Brain	Gray substance	(+)	+	+
	White substance	+	+	+
Spinal cord	Gray substance	+	+	++
	White substance	+	+	+
Eyes	Lens	++++	++++	++++
	Light-sensitive elements of the retina	++	(+)	++

(Table continued on Page 113)

(Table continued from Page 112)

Organs and Tissues	Amphibians (Axolotls, Frogs)	Birds (Roosters)	Mammals (Guinea Pigs, Rats)
Connective tissue of different organs (cells and fibers)	0	0	0
Cartilage of the breast	(+)	(+)	+
Erythrocytes	(+)	+	+

Note: Conventional symbols in the table: ++++ very intense reaction, +++ intense reaction, ++ moderately intense reaction, + weak reaction, (+) very weak reaction, 0 lack of any noticeable reaction.

Conclusions

1. The tryptophane content is different in a number of organs of birds and even in individual parts of organs.

2. The organs of chicken embryos of 6, 7, and 8 days' development are poor in tryptophane, and differences in its content in different organs are far less marked than in mature individuals.

3. Marked differences in the tryptophane content in the proteins of the organs of adult birds which were established in the process of differentiation of the organs, provide evidence of organospecific uniqueness of proteins.

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CHROMOSOME COMPLEX OF THE USSURI GEOGRAPHICAL RACE OF WILD MULBERRY SILKWORM MOTHS BOMBYX MANDARINA M. IN CONNECTION WITH THE ORIGIN OF THE DOMESTICATED MULBERRY SILKWORM MOTH BOMBYX MORI L.

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Studies conducted by the Japanese cytologists Yatsu and Kawaguchi established that the haploid number of chromosomes in the domestic mulberry silkworm moth Bombyx mori L. is 28 (Yatsu, 1913; Kawaguchi, 1928). The same research workers established the number of chromosomes in the wild species Bombyx mandarina M, related to the domesticated mulberry silkworm moth, a species erroneously assigned by the entomologist who had described it, Moore (1872), to the neighboring genus Theophila and which had been known for a long time in literature under the name Theophila mandarina M. The number of chromosomes in Bombyx mandrina turned out to be 27 by their data. Kawaguchi also studied the spermatogenesis and the ovogenesis of hybrids of the first generation, readily obtained through reciprocal crossing of Bombyx mori x Bombyx mandarina. At this time it was discovered that one large chromosome of the 27-chromosome complex of B. mandarina usually conjugated with two elements of ordinary size from the 28-chromosome complex of B. mori, forming a tetrad which is readily distinguishable by its size and triangular form in spermatogenesis and in irregular chromosome "with branching" in ovogenesis.

Inasmuch as B. mandarina, as Sasaki (1898) noted, can quite reliably be regarded as the "wild ancestor" of the modern domestic B. mori, Kawaguchi stated the natural assumption that when the domestic species was isolated there occurred a fragmentation of one of the 27 chromosomes of the wild ancestor and the haploid number rose by one chromosome.

The presence of 28 chromosomes in B. mori was confirmed by numerous authors who worked with material of the most diversified origins (breeds) and at present there can be no doubt that the number 28 is characteristic of all, without any exception, of the numerous races of domestic mulberry silkworm moths if one does not count the experimentally obtained polyploidic parthenogenetic clones (Astaurov, 1940, 1955, 1956) and other experimental polyploids (Hashimoto, 1933, 1934; Kawaguchi, 1936, 1938, and others).

As for further studies of the wild species, besides those already cited, we do not know of any later cytological studies, and therefore in subsequent experimental work with B. mandarina, for example in the work of Bobrov on hybridization of B. mori and B. mandarina (1936) and in the experiments of Astaurov and Ostryakova-Varshaver on heterospermatic androgenesis in these species (1957a, b) it was accepted that the haploid number of chromosomes in B. mandarina is 27.

Along with this, we must not forget that the Japanese authors made cytological studies only of B. mandarina of Japanese origin. Kawaguchi reported that he collected his material on the islands of Hokkaido and Kyushu. Although Yatsu did not directly indicate the origin of his material, one cannot doubt that here also he was dealing with material collected on the Japanese islands. In the meantime, the species B. mandarina has an extensive area of propagation and, in addition to the Japanese islands, inhabits extensive areas of the Asiatic continent, beginning with the latitude of Lake Khanka in Primorskiy Kray and further south to all of southeastern China, Indochina, clear to northern India. Therefore, one cannot exclude the possibility that different geographical races of B. mandarina could display differences in cytological respects. Unfortunately, we were not able to familiarize ourselves with the work of Ishihara (1943) which could be of great importance in the questions discussed here. However, judging by references, this work did not contain cytological data.) A. S. Bobrov discovered that when B. mori and Ussuri B. mandarina were hybridized no deviations from the normal in respect to fertility and viability of the hybrids were observed, which was noted by Kawaguchi when Japanese material was crossed. These genetic data caused Bobrov to state the assumption, not verified cytologically, that the Ussuri race of B. mandarina might have 28 chromosomes like B. mori, not 27 chromosomes. The possibility showed by Astaurov and Ostryakova-Varshaver (1927 a, b) of obtaining complete heterospermatic androgenesis in androgenic hybrids of the type B. mori (cytoplasm) - B. mandarina (nucleus) and B. mandarina (cytoplasm) - B. mori (nucleus) when the Ussuri race of B. mandarina was used, encouraged us to study this material cytologically.

Material and Methodology

A mass laboratory line from which larvae were taken to determine the number of chromosomes had its origin in material collected in 1951 and 1953, in Khasanskiy Rayon of Primorskiy Kray in the form of wintering egg masses laid on branches of wild mulberry trees. Later these mass lines were fed twice a year directly on mulberry trees (in gauze isolators), during the spring generation on plantations of the Georgian Institute of Sericulture (Tbilisi) and during the second summer-autumn generation on a plantation of the Kropotovsk Biological Station of the Institute of Animal Morphology, AN, USSR (Moscow Oblast, Kashirskiy Rayon). The Semenniki [Literally spermaries] subjected to study were taken from 20 caterpillers of the spring generation of 1956 in the beginning of the V [Meaning not clear, possibly May] age. The fifth abdominal segments of the caterpillers containing the spermaries, which had been removed for study, were fixed for a day in San Felice's fluid, then prepared from the surrounding tissue of the spermaries after the usual inclusion in parrafin and preparation of sections of 6-7 microns thick, then stained with iron hematoxylin (Heidenhain's stain). The haploid number of chromosomes was counted in metaphasal plates of spermatocytes of the first and second orders with an immersion object lens (90X) and eyepiece (15X) with the aid of a drawing apparatus. The diploid number of chromosomes in metaphasal plates of spermatogonii was not counted since such counts are very complicated due to the large number, tiny sizes, and exceedingly dense distribution of certain chromosomes.

Results

The first cytological studies revealed a divergence from the data obtained by Japanese authors on Japanese materials. The number of chromosomes in the metaphasal plates of spermatocytes of the first and second orders turned out to be 28, that is, the Ussuri race of B. mandarina showed a coincidence in this respect with the domestic silkworm moth B. mori, not with the 27-chromosome Japanese subspecies of B. mandarina (refer to the figure).

The lack of agreement in results obtained by us and by Japanese authors on material of different geographic origin but belonging to the same systematic species naturally compelled us to desire a particularly painstaking count of the number of chromosomes in an adequate quantity of material. In this connection, the making of preparations, their study and counting the number of chromosomes, were conducted independently and in parallel with two specimens. The results obtained from this procedure are presented in the table. The table shows that of 20 individuals studied (39 spermaries--in one individual only the right

spermary was present) 8 were suitable for counting the metaphasal plates in spermatocytes of the first and second orders. The remaining 12 individuals yielded a picture of earlier stages of spermatogenesis. A total of 127 plates was counted and the number of chromosomes in all of the plates, without exception, turned out to be 28.

The available material on the cytology of B. mandarina, probably the "wild ancestor" of the domesticated B. mori L., is still undoubtedly too scanty to permit general conclusions on the genetic and philogenetic interrelationships of these species. The differences discovered in the number of chromosomes in the Ussuri continental and the Japanese insular races of B. mandarina indicate that rather than passing on to similar generalizations, it is essential to establish the chromosome number of B. mandarina in different geographic points of its extensive habitat and, in particular, in those regions of southeastern China (the provinces of Shantung, Szechuan, and Hupei) and northeastern India (Bengal, and Assam) which can at present be considered probable centers of the origin of the domestic mulberry silkworm moth.

Number of Individuals	Spermaries Studied		Presence of Metaphasal Plates in Spermatocytes		Number of Counted Plates with 28 Chromosomes		Number of Plates with Different Numbers of Chromosomes	
	a	b	a	b	a	b	a	b
1	+	+	-	-	-	-	-	-
2	+	+	-	+	-	4	-	-
3	+	+	-	-	-	-	-	-
4	+	+	-	-	-	-	-	-
5	+	+	-	-	-	-	-	-
6	+	+	-	-	-	-	-	-
7	+	+	+	+	16	5	-	-
8	+	+	-	-	-	-	-	-
9	+	+	-	-	-	-	-	-
10	+	+	+	-	1	-	-	-
11	+	+	+	+	15	1	-	-
12	+	-	-	-	-	-	-	-
13	+	+	-	+	-	2	-	-
14	+	+	-	-	-	-	-	-
15	+	+	-	-	-	-	-	-
16	+	+	+	+	14	5	-	-
17	+	+	-	-	-	-	-	-
18	+	+	+	+	2	46	-	-
19	+	+	+	+	9	7	-	-
20	+	+	-	-	-	-	-	-
				Total	57	70	0	0

Note: a - right spermary; b - left spermary; + yes, - no.

In the meantime, we were able to make a cytological study of one male obtained in the first generation of crossing the Ussuri and the Shanghai races of B. mandarina. The number of chromosomes in divisions of the cpermatocytes of the first order, with a count of 14 metaphasal plates, turned out to be 28, from which one can conclude with conviction that the parent of the Shanghai race also had 28, not 27 chromosomes.

The discovery which has already taken place of a continental race of B. mandarina with 28 chromosomes permit one to consider that the previous hypothesis advanced by Kawaguchi (1928) to the effect that the domestic B. mori with 28 chromosomes came from the B. mandarina with 27 chromosomes and in which there occurred fragmentation of one of the chromosomes is very improbable. It is far easier to assume that in the probable centers of origin of sericulture B. mandarina also turns out to have 28 chromosomes, that B. mori has retained the number of chromosomes (28) inherent in the wild ancestor, and that, on the contrary, the isolation of B. mandarina on the Japanese islands had the consequence of forming an insular race with 27 chromosomes as a result of the association of two chromosomes of the ancestral continental form. The cytology of lepidopterons contains many examples in which separate races within the limits of the same species (for example, in Phragmatobia fuliginosa, in Solenobia pineti, in insular and continental races of Biston hirtaria, in laboratory lines and the basic forms of Philosamia cynthia and others) differ from each other in the number of chromosomes by one or two elements. In some cases it is more probable to assume that these differences are the result of fragmentation, in others, on the contrary, by association of chromosomes of the original ancestral form (refer to the article by Belyaev (1930) which was deovted to the phylogenesis of chromosome numbers in butterflies).

Conclusions

1. It was established that the southern Ussuri geographical race of wild mulberry silkworm moths Bombyx mandarina M., like the domestic mulberry silkworm moth Bombyx mori L., has a chromosome number of 28, differing in this respect from the Japanese insular race B. mandarina M. which has 27 chromosomes.

2. The previous assumption (Kawaguchi, 1928) that the domestic mulberry silkworm moth originated out of the 27-chroomosome ancestral form should be considered improbable. It is more probable that the wild ancestor of the domestic silkworm moth had, like the modern B. mori, 28 chromosomes and that when B. mandarina was settled on the Japanese islands the isolation was accompanied by the formation of a race with 27 chromosomes, at the same time two chromosomes of the initial species formed one large one by association.

3. To verify the assumptions stated here it would be very desirable to establish the chromosome numbers for the largest possible number of local populations of B. mandarina through the entire habitat of this species, particularly in the probable centers of origin of the domestic mulberry silkworm moth.

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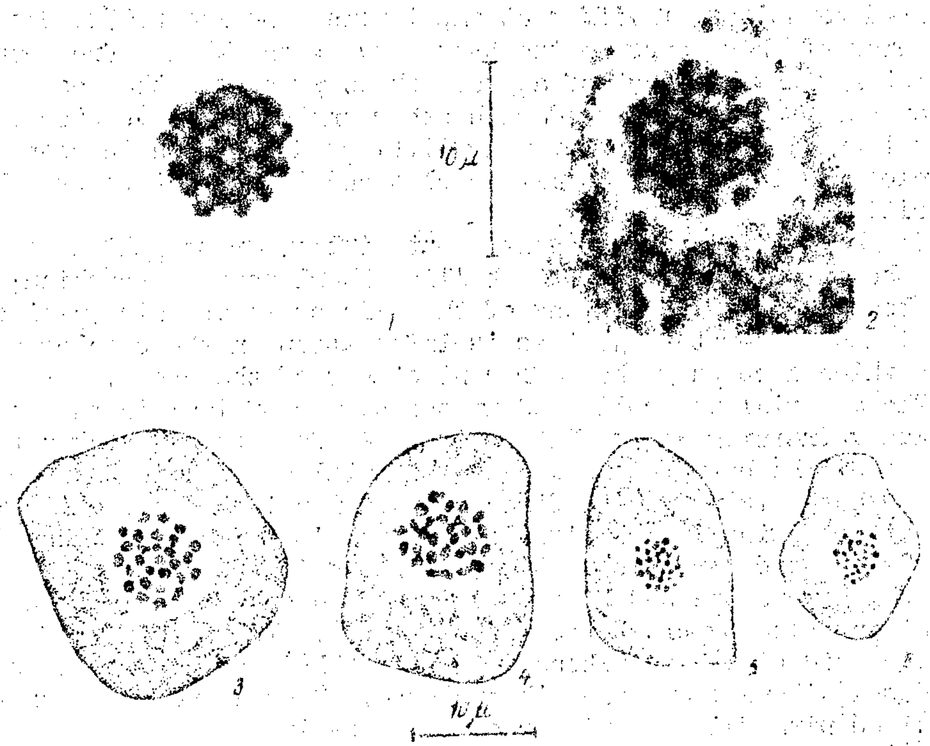
FIGURE APPENDIX



10 microns

1 2

3 4 5 6

10 microns

Spermatocytes of first (I) and second (II) order of Bombyx mandarina M.
1, 2 - spermatocytes of the first (I) order (photograph);
3, 4 - spermatocytes of the first (I) order (drawing, objective 90 X, eyepiece 15 X); 5, 6 - spermatocytes of the second (II) order (drawing, objective 90 X, eyepiece 15X).

A CYTOLOGICAL CHARACTERIZATION OF PROTOOPALINA CAUDATA ZELLER

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Opalinidae, which belong to the genus Protoopalina, are widely distributed parasites of different amphibians. Banina (1952) described four species of this genus in the territory of the USSR. Protoopalinidae are of interest in the study of a group which possesses the most primitive body structure as compared with other opalinidae. Species of the genus Protoopalina are comparatively little studied both in respect to life cycles and in respect to life cycles and in respect to cytochemical peculiarities.

In this article are presented material on Protoopalina caudata Z. from the intestines of the red-bellied Zherlyanka [batrachian] Bombina bombina L. Descriptions of the life cycle of this species are lacking in the literature just as is information on the cytological patterns which accompany the different stages of the cycle.

The material was collected in the summer of 1957 in the territory of the Kanev Training Forest Enterprise. P. Caudata were taken from the intestines of the batrachians and the material was examined under the microscope both living and in fixed preparations. Nucleic acids were studied after fixing in Kelly's fluid, Carnoy's fluid, and Schaudinn's fluid. Smears and paraffin sections prepared in the usual way 5 microns thick were stained by Feulgen's method with methyl green pyronin and toluidine blue by Brachet's method (1953). The presence of desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) was controlled with the aid of desoxyribonuclease and ribonuclease. Smears and sections were also stained with Heidenhain's green hematoxylin.

The proteins were studied in sections by staining them with mercury bechloride solution of bromphenol blue by the method of Mazia, Brever, and Alfert (1953). Histones were determined by the method of Alfert and Geschwind (1953).

Fat inclusions were studied in total preparations after fixation with 4 per cent neutral formalin and staining with a saturated solution of Sudan III and black Sudan B. Study of polysaccharides was conducted on paraffined sections 5 microns thick after being fixed with Carnoy's fluid and a 10 per cent solution of neutral formalin. The sections were stained with Bauer's method and with the aid of the ShIK (Schiff iodic acid) reaction by Hotchkiss' method (1948) with the use of KJO_4 in HNO_3 as an oxydizer. Under field conditions total preparations were stained with Lugol's solution. Saliva and diluted alkali solution served as a control when staining polysaccharides. Acid polysaccharides were studied by Hale's method (Hale 1946), the standard method of Pearse (1956)

and that of Hess and Hollander (1947). Mitochondria were displayed on the sections after fixing with Champy's fluid and stained by the Kull [transliterated] method.

Drawing were made with the aid of a drawing apparatus with MBI-1 and MBI-2 microscopes.

Protoopalina caudata Zeller are an ordinary, frequently-encountered parasite of the intestines of the red-bellied Zherlyanka. Two morphologically different forms are characteristic of this species. These forms were described by Metcalf (1923). One of them is the forma attenuata Metcalf, the second is the forma lata Metcalf. As a rule, one form predominates in a single population of protoopalinidae, but one can always find in any population a number of transitional individuals and a small number of individuals of the other form.

The form attenuata Metc. (Figure 1) is distinguished by a lengthened, spindle-shaped body. There is a well-marked caudal appendage on the rear end of the body. The dimensions of the protoopalinidae are small and can be given by the following figures: length of the body 85-120 microns, width of the body 25-53 microns, length of the nucleus 9.9-11.8 microns. The nuclei were round or slightly oval, at times connected by a neck.

The forma lata Metc. (Figure 2) differs from the preceding form by a wide, oval body which is tipped at the rear end with a caudal appendage. The nuclei are circular or oval, frequently connected by a fine neck. The following dimensions are usual for this form: length of the body 100-145 microns, width of the body 40-100 microns, length of the nuclei 6.6-11.7 microns, and the width of the nuclei 6.6-9.9 microns.

The life cycle of P. caudata proceeds along a course usual for the opalinid type and has much in common with the cycle of P. intestinalis Stein (Metcalf, 1923) and also with Opalina ranarum (Ehrenberg) (Konsuloff, 1922); Sukhanova, 1953). The life cycle of P. caudata is closely connected with the cycle of its host and in the spring, when the batrachians begin to spawn, the protoopalinidae also begin to multiply. During this period the protoopalinidae begin to divide intensively and form tiny precystic individuals. The latter were, for the most part, individuals with one nucleus (Figure 3) and were outwardly completely identical for both forms of P. caudata. The dimensions of the precystic individuals were as follows: length 20-35 microns, width 12-20 microns. All the precystic individuals possessed lengthened, spindle-shaped bodies with a rounded front end. In fixed preparations they had most frequently of all the form of a comma. Their encystment took place in the lumen of the rectum.

The cysts were rounded with a thin, transparent coating (Figure 4). The diameter of the cysts was 13-20 microns. In the spring there are usually exceedingly many cysts in the intestines of the batrachians and they are expelled into the water with fecal masses. The tadpoles are infested with the cysts and the copulation of the gametes and the further development of the zygotes up to fully formed individuals take

place in their intestines. The period of cyst formation in protoopalinidae is not wholly completed by the time the batrachians have finished spawning. During the summer the batrachians continue to remain in bodies of water and it is always possible to find a small quantity of protoopalinid cysts in their intestines. In the second half of the summer, also in the autumn and the winter, typical vegetative individuals, among which one can always find dividing individuals, predominate in the protoopalinid populations.

In the structure of the cytoplasm and the nuclear apparatus P. caudata has much in common with other species of protoopalinidae. All protoopalinidae are binuclear and both nuclei are identical in dimensions and form. The distinguishing feature of the nuclei of P. caudata is the fact that they are exceedingly weakly stained by Feulgen's method (Figure 5). The weak Feulgen reaction makes it possible to judge that an insignificant amount of DNA is contained in the nuclei, in contrast to the nuclei of many other protozoans, for example, infusoria of the genus Balantidium and Nyctotherus, which are likewise parasites in batrachian rectums.

An analogous result from the Feulgen reaction was obtained in Opalina ranarum (Konsuloff, 1930; Sukhanova, 1953), but when compared with its nuclei, the nuclei of P. caudata are still more weakly stained. The nuclei of the vegetative individuals have a diffuse coloring and only in some of them can one notice a few very small granules which have a more intense violet color. A unique peculiarity of the nuclei consists in the fact that a positive Feulgen reaction was discovered only in the centers of each nucleus while the peripheral portions remained colorless (Figure 5). It follows from this that the peripheral layer of the nuclei does not contain DNA and it is localized only in the central part of the nuclei.

In contrast to the vegetative individuals, the nuclei of the precystic forms and the cysts are characterized by a stronger degree of staining by Feulgen's method (Figure 6). The DNA in them is also located only in the central part of the nuclei, but here the bright violet granules of small size and distributed throughout the entire stained portion are distinctly visible. The intensity of staining of the nuclei in precystic individuals and cysts are more or less alike. Stronger Feulgen reactions in the nuclei of precystic individuals and cysts were also discovered when studying Opalina ranarum (Sukhanova, 1953).

The endoplasmatic granules, which are numerous in P. caudata, do not show a positive Feulgen reaction.

The results obtained from studying the nuclei with the nuclear reaction agree well with the results obtained by staining with methyl green-pyronin by Brachet's method. Methyl green stains only the center of the nuclei and the degree of staining is very weak (Figure 7), which may serve as still another proof of the presence of a small amount of DNA in the nuclei. Somewhat stronger staining by methyl green was characteristic of the nuclei of precystic individuals and cysts, a result which

was also obtained by the Feulgen method. Judging by intensity of staining, the nuclei of precystic individuals and the cysts contain more DNA than the nuclei of the vegetative forms of protoopalinidae. It is quite difficult to establish the causes of such differences in DNA content, but, in all probability, the period of spring cyst formation, which is connected with intensive physiological activity, is also connected with increased synthesis of DNA.

The use of methyl green-pyronin also permitted discovery of the nature of the peripheral parts of the nuclei which were not stained at all by Feulgen's method. It turned out that in all nuclei the border zone was stained exceedingly intensely with pyronin to a bright red color with a small mixture of a violet shade (Figure 7, 8). When stained with Heidenhain's iron hematoxylin (Figure 9) these parts became entirely black, while the centers of the nuclei had a quite weak gray color.

The strong pyroninophilia, the intensity of staining with iron hematoxylin, the lack of a positive Feulgen reaction--all these make it possible to draw a conclusion concerning the nature of the peripheral granules and the small granules in the center of the nuclei. Their nucleolar nature we also confirmed by the use of toluidine blue by Brachet's method (Figure 10). In the sections prepared with the aid of the latter method, the nucleolar parts acquired a blue color while the entire center of the nuclei which contained DNA were quite weakly stained.

The use of the ferment ribonuclease, with which several preparations were processed, proved to be still another necessary confirmation of the nucleolar nature of the peripheral parts of the nuclei of the protoopalinidae. After being processed with ribonuclease, the nucleoli which contained RNA lost their capacity for being stained by pyronin and toluidine blue (Figure 11). Consequently, the nuclei of P. caudata are characterized by the presence of large nucleoli which contain RNA. In the dormant nuclei, in the majority of cases the nucleoli occupy the entire peripheral part, being distributed in the form of a solid belt, or a belt interrupted in several places. However, the nucleoli also may have the form of round or oval drops lying somewhat separated from the edges of the nuclei, sometimes even in the centers of the nuclei. Nucleoli are retained in the nuclei of precystic individuals and cysts and their form and distribution remain the same as in vegetating individuals.

The nucleoli are also retained in dividing nuclei (Figure 12). Here, as a rule, they acquire an extended form and lie in the peripheral part of the nuclei in the form of lengthened stria. Large nucleoli were also observed in the nuclei of Opalina ranarum (Sukhanova, 1953) and were similar in form and distribution to nuclei of P. caudata. There was much RNA in the nucleoli of both species of opalinidae. The RNA content of the nuclei was considerably higher than the DNA content. Therefore, in DNA and RNA content, the nuclei of P. caudata can be compared with the macronuclei of certain low marine infusoria, for example, Trachelocerca phoenicopterus Cohn. (Raykov, 1956). A small amount of DNA and a great

amount of RNA are contained in the complex macronuclei of this species. If one judges by the intensity of staining by Feulgen's method and by methyl green-pyronin, then the quantitative predominance of the latter becomes perfectly obvious. The same ratio of nucleic acids is inherent in the nuclei of P. caudata.

In protoopalinidae the localization of RNA is not limited to just the nucleoli of the nuclei. As a rule, the endoplasm also contains much RNA (Figures 7, 8, 10) and the strong basophilis of the plasm, when stained by methyl green-pyronin and by toluidine blue with Brachet's method, serve as proof of this. The ferment ribonuclease was used as a control in both methods of staining. The strongest pyronophilia was shown by the endoplasm of dividing individuals, precystic forms and cysts; while the vegetative individuals contained a bit less RNA. On the whole, however, there was considerably more endoplasmatic RNA in P. caudata than in Opalina ranarum from grass frogs. This comparison is extremely rough since it was possible solely on the basis of differences in intensity of staining of the endoplasm with toluidine blue and methyl green-pyronin. The presence of a large quantity of RNA in protoopalinidae indicates its important physiological role.

Endoplasmatic bodies do not show a positive reaction to RNA.

The proetin components of the cytoplasm and the nucleus were also studied in P. caudata. General staining of proteins was accomplished with a mercury bichloride solution of bromphenol blue (Figure 12). In the stained sections the cilia were distinctly visible, the ectoplasm was intensely stained, while the endoplasm had a weak blue color and a homogeneous structure. The endoplastmatic bodies, particularly their membranes, were stained a bright blue color. The reaction with bromphenol blue revealed the protein nature of the endoplasmatic bodies. Both protoopalinid nuclei displayed the same degree of staining, and in each of them the central part containing DNA had a pale blue color but the nucleoli were very intensely stained.

Of the separate protein components, the histones were subjected to study. The method of staining with fast green by the method of Alfert and Geschwind (1953) was used for discovering them. The localization of histones in the cell coincides essentially with the localization of nucleic acids. Therefore, in protoopalinidae fast green stains both the nucleus and the endoplasm (Figure 14). The central part of the nucleus which contains DNA was weakly stained and the nucleoli were stained a bit more strongly. The endoplasm acquired a green color, while only the endoplasmatic bodies remain colorless in it. The ectoplasm was not stained at all.

The stored nutritional substances whose basic place of localization is the endoplasm are of great interest to research. In P. caudata the stored nutrient substances are represented by neutral fat and polysaccharids, of which glycogen predominates. The neutral fat is distributed in the endoplasm in the form of drops which are well stained with Sudan III and black Sudan B (Figure 15). Stores of neutral fat were present at all stages of the life cycle but the greatest quantity

was observed in the early spring. In addition to staining the drop of neutral fat, Sudan black B also stains the lipoids contained in the different organoids. Therefore, when total preparations were processed with this stain, the pellicle, endoplasmatic bodies, and the nuclear membrane (very weakly) were displayed.

Polysaccharides were readily discovered by the ShIK reaction (Figure 16). The polysaccharides were most frequently displayed in the form of numerous fine granules lying in the endoplasm. However, the endoplasm could also be stained diffusely, which was particularly characteristic of spring protoopalinidae. Diffuse staining of the endoplasm indicated an insignificant quantity of polysaccharides. A very weak, scarcely noticeable positive ShIK reaction was characteristic of the ectoplasm.

Staining by Bauer's method revealed some glycogen so that one might speak only of traces of it. Control with saliva showed that the sections subjected to the action of saliva still retained a positive, even though weak, ShIK reaction. This fact makes it possible to assume that other polysaccharides were contained here in addition to glycogen. In order to discover the nature of these polysaccharides, they were tested by a reaction for acid polysaccharides. Hale's method did not yield positive results, but the standard Pearse method made it possible to display a few fine metachromatic granules stained to a bright red color. In all probability, we have here substances of the mucopolysaccharide type.

On the whole, however, P. caudata contained very little of the polysaccharides, as contrasted with Opalina ranarum, in which the ShIK reaction and the Bauer reaction yielded bright staining of the endoplasm.

The endoplasmatic bodies did not show a positive reaction to polysaccharides in a single case. These bodies were distributed throughout the entire endoplasm, the amount of them was quite large (up to 200 and more per individual), but the length of each body was 2-2.5 microns. As seen from the preceding description, the endoplasmatic bodies showed a positive reaction to protein, were intensely stained by iron hematoxylin, and their membranes contained lipids (Sudan black B). However, the most interesting result was obtained by using the Kull method for staining mitochondria. In the sections stained by this method the endoplasmatic bodies acquired a bright red color characteristic of mitochondria (Figure 17). Therefore, the Kull method, as compared with other methods of staining, made it possible to draw a conclusion as to the mitochondrial nature of the endoplasmatic granules. The latter are present in the endoplasm of all species of opalinidae and are retained in all stages of the cycle.

Different views exist regarding the endoplasmatic granules and their role in the life activities of opalinidae. Thus, Metcalf (1909) considered that the composition of "endoplasmatic spherules" was close to that of paraglycogen. Konsuloff (1922, 1930) suggested that these

granules are a fragmented macronucleus. Zasukhin (1925) correctly pointed out that the endoplasmatic granules were formations of a type of mitochondria. When the life cycle of Opalina ranarum was studied, it was possible to establish that the endoplasmatic granules were actually an organized form mitochondria (Sukhanova, 1953). This was also confirmed for P. caudata. The fact that the granules of endoplasm play an important role in the life activities of opalinidae is indicated by their large quantity in every species and also by the fact that they are present in all stages of the life cycle.

Conclusions

1. Protoopalina caudata Zeller is a usual and frequently-encountered parasite found in the intestines of the red-bellied Zherlayanka (Bombina bombina L.). The life cycle of this species proceeds along a course that is common to the opalinid type and includes a period of intensive mutliplication which essentially coincides with the spawning period of the batrachians.

2. Both nuclei of the P. caudata are identical in size, shape, and structure. The nuclei contain an insignificant amount of desoxyribonucleic acid (DNA) and much ribonucleic acid (RNA) which is localized in large nucleoli. A large amount of RNA is also contained in the endoplasm of P. caudata.

3. When proteins were studied with a mercury bichloride solution of bromphenol blue, a positive reaction displayed all the organoids of the protoopalinidae. At this time the endoplasmatic bodies were especially intensely stained. The nuclei and the endoplasm of P. caudata yielded a positive reaction to histones.

4. Stored nutrient substances were represented by neutral fat and polysaccharides. There was very little of the latter in P. caudata.

5. The endoplasmatic granules of P. caudata represented, by their nature, an organized form of mitochondria.

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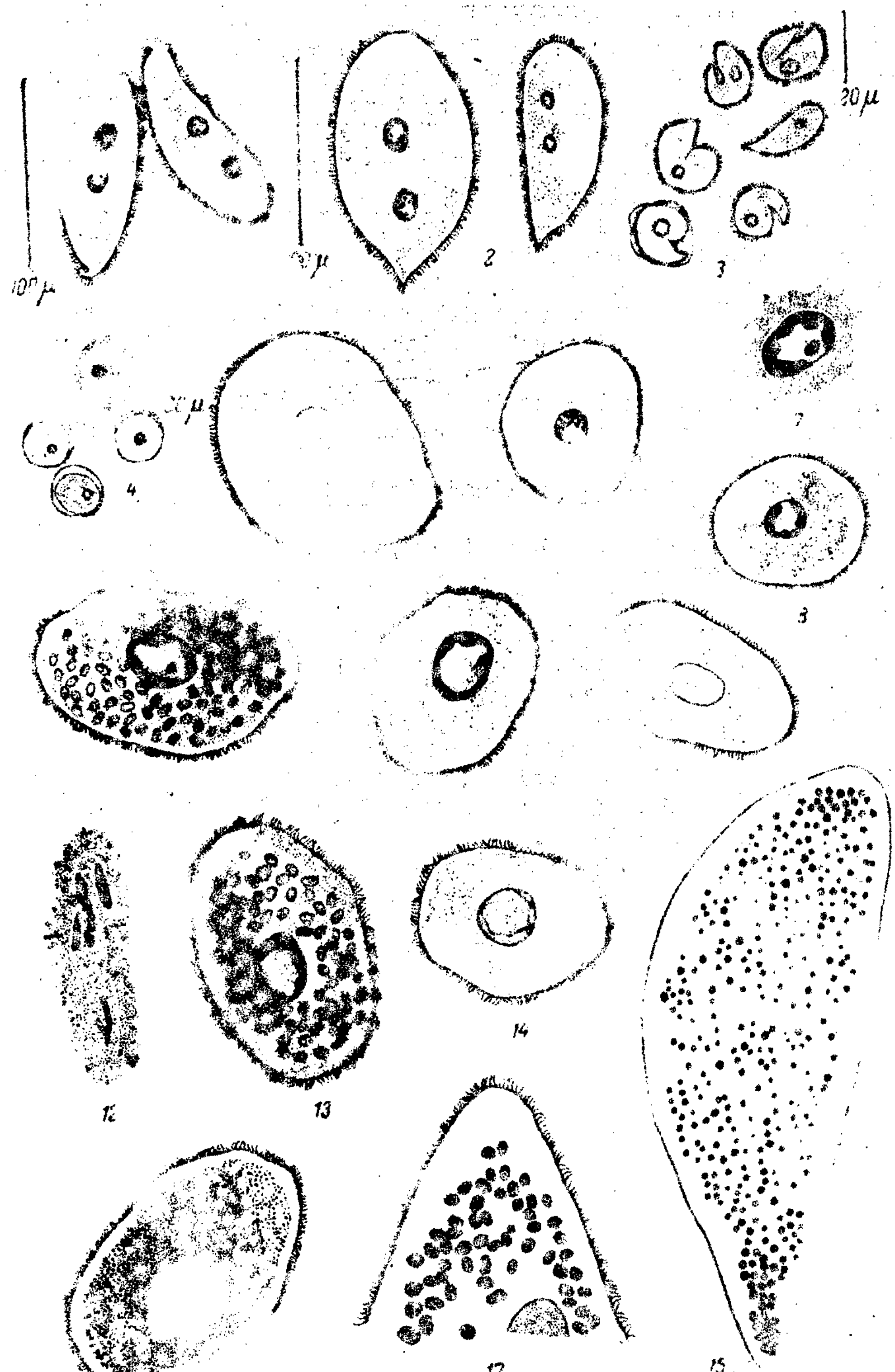
FIGURE APPENDIX



100 microns 100 microns 20 microns

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16 17 15

Figures 1-17.
1. Protoopalina caudata f. attenuata Metc. (iron hematoxylin of Heidenhain).
2. P. caudata F. lata Metc. (Iron hematoxylin of Heidenhain).
3. Precystic forms (iron hematoxylin of Heidenhain). 4. Cysts (iron hematoxylin of Heidenhain). 5. Nuclei of vegetative individuals stained by Feulgen's method. Only the center of the nucleus is weakly stained. 6. Nuclei of precystic forms. Same staining. Fine Feulgen-positive granules are visible in the center of the nucleus, the cytoplasm is colorless. 7.-Nucleus of a vegetative individual. Stained with methyl green - pyronin. 8. Precystic form. Same staining. 9. Vegetative individual. Stained with toluidine blue. 11. Vegetative individual processed with ribonuclease. Stained with methyl green - pyronin. 12. Nucleoli in a dividing nucleus. Same staining. 13. Vegetative individual. Stained with bromphenol blue. 14. Vegetative individual. Stained with fast green. 15. Fat in P. caudata. Sudan black B. 16. Vegetative individual. ShIK reaction. Quite fine granules stained in the preparation to a pale violet color are distributed throughout the entire endoplasm. 17. Vegetational individual. Mitochondria stained by Kull's method. In the preparation the mitochondria are stained to a bright red color. 1 - 2 objective 20X, eyepiece 10X; 3 - 4 objective 40X, eyepiece 10X; 5 - 15 objective 90X, eyepiece 10X.

BOOK REVIEW

G. Yu. Belitskiy Ionnye mekhanizmy osnovnykh nervnykh protsessov

[Ionic Mechanisms of the Basic Nervous Processes]. 174 pages, 47 illustrations. Medgiz [Gosudarstvennoe izdatel'stvo meditsinskoy literatury - State Publishing House of Medical Literature] Leningrad, 1958.

G. Yu. Belitskiy's book nature of excitation and inhibition, a very important problem both for the theory of physiology and for medicine. It sets forth his own theoretical ideas on these basic processes and presents experimental material (his own and from the literature) to support them. The book is divided into seven chapters with a brief introduction and conclusion.

The physiological significance of the ionic gradient between the cell and the medium is discussed in the first chapter. The author believes that unequal distribution of ions between the cell and the medium (chiefly potassium and sodium) constitutes the "most important principle of construction of living tissues." In the author's opinion, the enormous physiological importance of the ionic gradient consists in the following: in the first place, its presence creates a concentrated potential difference and thus serves as a source of electromotive forces; in the second place, the physicochemical basis of excitation is an exchange of intracellular potassium for the sodium of the medium, which leads to equalization of the ionic assymetry. Therefore, the tissue can be excited only in the presence of a definite steepness of the gradient. If the gradient is reduced or entirely removed, then excitability also drops and the potential difference is likewise reduced.

Reduction or entire removal of ionic asymmetry, in the author's opinion, can be achieved artificially, for which purpose the tissue should be placed in detritus of the very same tissue, then the cells and the medium surrounding them will be, so to speak, identical. Such experiments conducted by the author on muscle and nerve tissue showed that when the tissues were placed in detritus they developed unexcitability quite rapidly, contractures occurred in the muscle tissue, and the quiescent current decreased. In this case, according to the author, excitability drops, not because the tissue itself was changed when it was placed in detritus, but because the ionic gradient was removed. The author writes: "By artificially removing the ionic assymetry we have, so to speak, freed the tissue from the necessity of doing work to support the heterogeneity of the biological system" (page 21). Starting from these considerations, G. Yu. Belitskiy believed that isolated tissue

placed in detritus should survive a longer period than in Ringer's solution. The experiments conducted to verify this hypothesis did not yield clear results. The author hopes that in the future, with more complete mastery of methods for removing ionic assymetry, it will be possible to increase the periods of survival of tissues.

The author thinks that the experimentor may be able artificially not only to weaken but to strengthen ionic asymmetry. For this purpose the tissue should be placed in hypertonic NaCl solution. Strengthening of asymmetry would occur, on one hand, because the sodium content of the medium would be increased, and on the other hand the concentration of intracellular potassium would be increased due to loss of water in the cell to the hypertonic solution. Strengthening of the ionic asymmetry which exists in the norm should be accompanied, according to the author, by increased excitability and greater electrical potential in the tissue.

The next three chapters set forth in detail the author's point of view on the role and importance of the three basic cations (Na, K, Ca) in physiological processes. When the cells are in a state of rest the sodium ions are indifferent to excited tissues; their role amounts to maintaining the osmotic relationships between the cell and its medium. Sodium ions do not participate in the creation of quiescent potential, they can affect the quiescent potential only indirectly by changing the electric conductivity of the medium thereby decreasing or increasing collateral circuits in the tissue. Under excitation, however, the sodium ions penetrate into the cell in exchange for potassium ions, thereby taking active part in the process of excitation.

G. Yu. Belitskiy ascribes enormous importance to the role of potassium in physiological activities--the potassium ions participate in the process of excitation and in the process of inhibition. A concentrating gradient of potassium also creates quiescent potential. As a concrete mechanism which causes this concentrating gradient the author assumes either a semipermeable membrane or anionic complexes of protoplasm which selectively combine the potassium ions, as follows from Ling's theory. For G. Yu. Belitskiy the important thing is that in both cases potassium is electrochemically active and thus serves as a source of a concentrating potential difference.

Calcium, in contrast to sodium and potassium, is present in tissues in ionic and in combined forms and may readily change from one form to the other. Under certain circumstances the interaction between the calcium ions of the medium and the phosphates of the protoplasm lead to the forming of a boundary layer which possesses the properties of a membrane. This reaction proceeds like a type of precipitation reaction. If these reactions are restricted to an injured surface, then something like a polarized layer is reconstructed on the injured surface, or membranes, that is, the properties of a normal surface are restored. If, on the other hand, the precipitation reaction is extended to the depths of the muscle fibers, then one observes that the entire mass of

the cell is involved in the reaction. This is characterized by an irreversible loss of excitability of the muscle and its capacity for developing electrical potential. Such, in the general viewpoint of the author, is the significance of the basic inorganic ions in physiological processes.

The fifth chapter is devoted to ionic asymmetry in connection with patterns of electrical stimulation. The author believes that the passage of an electrical current inevitably reduces the ionic gradient and gives rise to a defensive reaction on the part of the living tissue countering a merging of the initial level of ionic asymmetry. The author considers that this is confirmed by an increase of resistance to the current in time (in several milliseconds the resistance increased several hundred times). A change in the level of ionic asymmetry to a definite value depending upon the quantity of electricity passing through tissue leads to a process of excitation. In this case, if the tissue has a low level of ionic asymmetry (like smooth muscle tissue or altered tissue), perversion of the normal electrotonic reaction occurs.

The bioelectrical gradient in the spinal cord and in the digestive tract of a frog are described in the sixth chapter. G. Yu. Belitskiy believes that it is possible, with the aid of external polarization, not only to strengthen or to weaken the bioelectrical gradient, but also to change its direction. Regulation of the electrical gradient can be utilized for therapeutic purposes.

In the final, seventh chapter, the author expresses some opinions on the general character of the relationships between the ionic asymmetry and the metabolism and repeats his viewpoint on the nature of the processes of excitation and inhibition.

Excitation is characterized by an ionic shift which merges the initial level of asymmetry of the living tissue toward physical equilibrium with the medium. The departure of potassium from the cells leads to its accumulation in the intercellular spaces and to a sharp reduction of the ionic gradient, at which time excitation is no longer possible and inhibition occurs.

Further departure of ions from the cell is stopped. This is a defensive reaction which protects the cell from an excessive and irreversible loss of the substances accumulated in it. However, inhibition can occur not only as a consequence of reduction of ionic asymmetry, but also as a result of making more difficult the exchange of ions (lack of sodium in the medium, the action of calcium ions, of the anode of direct current), even though there is an ionic gradient. The physicochemical basis of all types of inhibition are one in the sense that it always means the impossibility of exchange of ions between the cells of the excited tissues and their medium.

The factual data obtained by G. Yu. Belitskiy on the action of detritus on different tissues are of undoubted interest. However, it is difficult to agree with the author's treatment of this material.

We believe that it is impossible to change only the asymmetry in the distribution of substances between the cell and the medium (even though to a limited extent) without changing the state of the cell itself. Any changes in the distribution of substances are the consequence of changes in the state of the protoplasm. From our point of view, it is unthinkable that a living cell should exist without asymmetry; the latter is destroyed only as a result of damage (excitation) or death of the cell. Likewise, a reduction in excitability of tissues placed in detritius is a consequence of the influence of the detritus on the protoplasm itself, not a consequence of artificial removal of asymmetry, as the author believes. Judging by G. Yu. Belitskiy's own data, detritus is a strong stimulator and causes a local, stable excitation in tissues, like many other influences. In this case, the tissue loses excitability, muscular contracture occurs, and the quiescent current decreases.

In his constructions the author starts from the assumption that the potassium in the protoplasm is found in a free ionized state and accepts this as a proven fact. In reality, however, in recent times more and more data is accumulating in favor of the position that the basic part of the potassium of the protoplasm is combined and not ionized (Ernst und Fricker, 1934; Reginster, 1937; Steinbach, 1940; Hahn and Hevesy, 1941; Krogh and Lindberg, 1944; Kometiani and others, 1944, 1946, 1947, 1948a, b; Sent-Dzhord'i, 1947; Stone and Sapiro, 1948; Tron, 1952, Harris, 1953; Troshin, 1956, 1957; Ungar, 1957).

If one stays on the soil of facts, then it is essential to speak of the presence of asymmetry in the distribution of potassium, sodium, and other substances, but not their ions. There are excellent grounds for believing that the detritus should differ sharply in its ionic composition from the contained cells. The concentration of free potassium is greater in the detritus than in the cell. In the preparation of the detritus, when the tissue is mechanically ground and the protein complexes are destroyed, the ions, and first of all those of potassium, are freed in large quantities. From G. Yu. Belitskiy's point of view, however, mechanical damage to tissue had no effect on the status of the potassium, which, in his opinion, was in a free state in the cells and in the detritus. In the experiments whose results formed the basis of the theoretical constructions of the author, the problem was solved as to what happened to the excitability and biopotential of the cell, whether the ionic gradient was strengthened or weakened. But G. Yu. Belitskiy merely showed in his experiments that under the influence of the detritus excitability and the value of the quiescent current dropped while excitability and the value of the quiescent current increased under the influence of the hypertonic solution. However, the author did not prove that ionic asymmetry increased under the action of the hypertonic solution of sodium chloride. Moreover, it is very probable that when the cells were placed in a hypertonic solution of sodium chloride, the sodium content of the cell increased and the potassium content decreased.

In confirming that the electrical potential is increased with hypertonia, the author presents the curve of one of his experiments conducted on a frog sartorius muscle (page 24). According to this curve, a double Ringer's solution makes the tissue positive. This is contradicted by our experimental data (not published as yet), according to which hypertonic solutions always make [tissue] negative. It is a pity that the author did not indicate the total number of experiments and their mean value. Perhaps the data he obtained depend upon some sort of methodological error?

At the end of the last chapter G. Yu. Belitskiy discusses in considerable detail the problem of the localization and the origin of bioelectrical potentials. He considers that biopotentials occur due to a difference in the ionic composition of the cell and the medium, that they preexist and the basic jump in the difference of potentials takes place on the surface of the cell, that is, on a division of media which differ in ion concentration. In defending these two basic assumptions, G. Yu. Belitskiy started a polemic against the phasal theory of biopotentials (Nasonov and Aleksandrov, 1944), according to which biopotentials occur only when tissue is injured or excited. The author turns again to the facts which were utilized by Nasonov and Aleksandrov for criticizing the membrane theory of biopotentials, and tries to give new explanations for them which would not contradict the membrane concept. Thus, he thinks that the rejuvenating effect of an incision on skeletal muscles, which was shown convincingly once again in the work of D. N. Nasonov and V. Ya. Aleksandrov of 1950, takes place because the injured end of the muscle was soaked in a physiological solution for a prolonged time (3 hours) prior to receiving the repeated incision. This should have strengthened the negative influence of collateral circuits on the value of the quiescent current of the muscle, in connection with which cutting off the injured end of the muscle which had been soaked in the solution could also lead to an apparent increase in the quiescent current. In our opinion, this explanation is inadmissable, since in this work Nasonov and Aleksandrov conducted experiments in which the effect of renewing wounds (true, of smaller size) was obtained on a muscle which was kept under the conditions of a moist chamber so that the incision was not soaked in the solution.

Further, the author denies without any grounds the fact of an increase in the value of the quiescent current of the muscle immediately after the infliction of a transverse incision. He considers that this phenomenon can be observed only when two conditions are met: the muscles must be kept for a long time in a physiological solution before the experiment or be fatigued, and the contact of the muscle with the electrode placed on its transverse section must not be interrupted during the entire period of observation. Under these conditions, according to the author, a diffuse difference of potential occurs on the boundary between the physiological solution and the transverse incision, which is opposite in sign to the quiescent potential. In the course of the

first few minutes this diffuse difference of potential drops to half its initial value, thereby seemingly increasing the total potential difference. To note this effect, however, a constant contact between the transverse incision in the muscle and the electrode is necessary; if, on the other hand, the electrode is removed and applied again just before each measurement, this effect is not observed (unfortunately, the author does not present the details of these experiments). On fresh muscles, however, this effect cannot be observed because there is a very sharp drop in potential after the incision is made so that the increase in potential difference as a consequence of the decrease in the diffuse potential difference does not register against this background. Verifying experiments especially set up by us showed that the effect of an increase in potential immediately after the incision occurs even when conditions required by the author are not observed; the potential increases for fresh muscles too, and also when the contact between muscle and electrode is interrupted throughout the period of observation. If the author had been right to any degree in that changes in the diffuse potential registered here, then there should have been an increase in potential in the nerve, and in the heart muscle since the diffuse potential should have occurred there too. Therefore, the author should give an answer as to why this effect did not occur in the objects mentioned above.

G. Yu. Belitskiy also discusses facts connected with changes in the quiescent potential with the action of different agents on a transverse incision. He believes that nonelectrolytes (narcotics and sugar) increase the potential difference only because they eliminate collateral circuits which are formed by the contents of intercellular spaces that conduct electrical currents. Calcium, on the other hand, when applied to the incision, decreases the potential difference because it again restores a polarized layer on the incision. The other salts, in the author's opinion, do not exert any effect on the value of the potential difference. The data obtained in the Laboratory of Cellular Physiology of Leningrad University indicate the error of this assertion.

Based on the foregoing we believe that the author's basic conclusions are unfounded.

Many problems raised by G. Yu. Belitskiy merit the most serious attention, but their solution in the reviewed work is very questionable.

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NEWS ITEMS

INTERNATIONAL SYMPOSIUM (BRUSSELS) ON THE RELATIONSHIPS OF THE NUCLEUS AND THE CYTOPLASM

V. I. Vorob'yev

A symposium convened by the International Society of Cellular Biologists on the relationship between the nucleus and the cytoplasm was held in June 1958 in Brussels. Many well known Belgian, French, English, American, and West German cytologists and biochemists participated in the symposium.

The symposium was opened by J. Brachet (Belgium) who emphasized the importance of the problem of the relationship between the nucleus and the cytoplasm in modern cytology.

F. Leman (West Germany) presented a paper on the functional significance of the submicroscopic nuclear structures of amoeba and the mitotic apparatus of Tubifex embryos. The paper was accompanied by a demonstration of splendid electronic microphotographs of all stages of cellular division. The author followed the formation of a spindle with a very fine structure of threads. Leman emphasized that the picture observed in the electron microscope frequently depends upon the fixing agent selected and on the physiological state of the cells of the object. He pointed out the necessity for using different methods for producing preparations and for always studying a large series of preparations in different successive stages of functional activity of cells or in different stages of their development.

V. Bernard (France) spoke of electron microscope studies of different structural formations of the nucleus and the cytoplasm. The structure of the chromosomes and centrioles was studied in detail. Study of the nuclear membrane revealed that it consists of a double layer of granules. At the same time, the nuclear membrane is a part of the membrane of the ergastoplasm. New structures which were membranes consisting of two layers of granules were discovered in the cytoplasm. They are strongly basophile. Chains of granules containing ribonucleic acid were also discovered in the cytoplasm. Study of cancer and embryonic cells revealed a significant similarity in their submicroscopic organization. Cancer and embryonic cells differed from normal

mature cells, first of all, in the lack of differentiated structures of the ergastoplasm.

The largest number of papers was devoted to study of the biochemical aspects of the interrelationship of the nucleus and the cytoplasm. F. Jacob (France) spoke on the transfer of genetic information at the time of sexual conjugation in bacteria. He studied the process of conjugation between male and female lines of Escherichia coli. In the process of conjugation the chromosome material of the individual of the male line strikes into the individual of the female line. The genetic material in the zygote which forms at this time is integrated; then there proceeds a segreation of the chromosome material, and bacteria are formed which possess the genetic patterns of both sexual types. Transfer of genetic information was studied by a number of indices: the transfer of radioactive phosphate from the male line to the female line, the transfer of resistance to a phage, the transfer of the capacity to synthesize adaptive ferments.

The paper by V. Plaut [literal] (USA) was devoted to the synthesis of ribonucleic acid in the nucleus and the cytoplasm. Experiments were conducted on amoebae and their fragments with and without nuclei, basically by a microautoradiographic method. Exceedingly intense synthesis of RNA was observed in the nucleus. Facts were presented which indicated a more intense synthesis of RNA in the cytoplasm of fragments without nuclei as compared with the synthesis of RNA in the cytoplasm of fragments with nuclei.

The paper by J. Brachet (Belgium) contained new data in regard to the biochemical relationships between the nucleus and the cytoplasm in amobae and acetabularia. A decrease in intensity of growth of the parts without nuclei was accompanied by a reduction in synthesis of protein and RNA. Brachet's direct biochemical studies, in contrast to Plaut's results, revealed that while the specific activity of RNA in the fragments with nuclei and those without nuclei was the same, the total quantity of RNA being synthesized in the anuclear fragments turned out to be 40-50 per cent of the former. Data were obtained which indicated the unique chemical structure of RNA synthesized in the anuclear fragments of acetabularia. The nuclear fragments were more sensitive to certain poisons which inhibited protein, for example, chloramphenicol.

S. Peltz (USA) told of his experiments which revealed the important role of the cellular nucleus and its DNA in forming the keratin of the skin. The synthesis of keratin by the cells of the skin of a large embryo in a tissue culture was studied with the aid of cystine - S^{35}. He observed an intense inclusion of this amino acid in the nuclei of those cells which were believed to synthesize keratin. A definite correlation was noted between the ratio of amino acids in the histone fraction of the nuclei and the cystine content in [illegible]-keratin.

A. Fik [literal transliteration] (Belgium] studied the metabolic processes in chromosomes. Experiments were conducted on isolated chromosomes separated out of the nuclei of animal cells which were

previously injected with radioactive amino acids or nitrogen bases contained in nucleic acids. The microautoradiographic method was employed to determine the place of inclusion in the chromosome of C^{14} - phenyl-alanine, C^{14}-adenine and thymidine, tagged with tritium. It was discovered that phenyl-alanine is diffusely spread through the entire length of the chromosome. On the other hand, C^{14}-adenine was found chiefly in the basophile parts of the chromosome. Thymidine tagged by tritium is included ordinarily in the very same places as adenine. However, the distribution of thymidine is distributed unevenly in the basophile places of the chromosome--there are places which include the tagged thymidine with special intensity. It was noted that when the physiological state of the cell was changed, the intensity of inclusion of thymidine was also changed.

A. Mirsky (USA) devoted his paper to the relationship between the nucleus and the cytoplasm in connection with the synthesizing activity of the nucleus. The last data obtained in the author's laboratory were presented here in regard to synthesis of protein, RNA, and ATF [adenozintrifosfat-adenosine triphosphate, ATP] in isolated nuclei. It was shown that a certain optimum exists for total concentration of electryolytes for the synthesis of proteins on the order of 0.07 M. In this case K^+ and Na^+ are antagonistic--K^+ reduces the inclusion of tagged amino acids in protein while Na^+, on the other hand, stimulates this process. The synthesis of RNA in isolated nuclei was studied and it was discovered that the most intense synthesis went on in the nucleolus. Experiments showed that coenzyme I synthesized in the cytoplasm was necessary for synthesis of RNA.

The paper by Zh. Chayen [transliterated] (Great Britain) presented data on the quantitative chemistry of DNA in connection with the role that this compound plays in the physiology of cells and in heredity. Cytochemical determination of DNA was combined with the quantitative fractionation of the structures. Chayen observed changes in DNA in the cell during development. He believes that ideas of a constant DNA content in the nucleus are dogmatic and do not correspond to the modern level of our knowledge.

E. Wilson's (Great Britain) paper on the resistance of amoebae to antigens was devoted to adaptive phenomena which were studied by transplanting nuclei. The experiments indicated that the resistance of the amoebae depended not only on the nucleus, but on the cytoplasm, too.

The problem of the transfer of information from DNA to enzymes was discussed in a paper by A. Pardi [transliterated] (USA). He studied the synthesis of adaptive enzymes (β-galactosidase, d-serine-desaminase) in Escherichia coli under different injurious influences (for example, freezing and thawing). At this time the intensity of synthesis of adaptive ferments was compared with the tempo of synthesis of nucleic acids.

A paper by Kh. Shantren [transliterated] (Belgium) contained very interesting data on the effect of certain purine analogs which separate the synthesis of nucleic acids from the synthesis of proteins. The effect

of tagged C^{14} - azaguanine on the rate of synthesis of DNA, RNA, and protein in bacteria was studied. Addition of azaguanidine to a bacterial culture inhibited the synthesis ofprotein in as little as 10 minutes, while the synthesis of DNA began to decrease only in 60 minutes, and the synthesis of RNA was noticeably accelerated. It turned out that it was possible to select such small concentrations that the synthesis of DNA would no longer be inhibited, the synthesis of RNA would be accelerated as before, and the synthesis of protein would still be inhibited. It was shown that azaguanine enters into the composition of RNA. Guanosine reduces the inhibition of synthesis of protein by azaguanine, dislodging the latter from RNA.

M. Fishberg (Great Britain) presented the results of studies on the transplantation of nuclei in cells of amphibians in connection with the problem of potential properties of the nuclei of cells of differentiating tissues. He studied homospecific and heterospecific transplantations. At the same time he observed great variability in regard to further development of the cells. Transformations of the genetic material, of a different type of chromosome rearrangement, occurred frequently.

J. Moore (United States) studied the transfer of cellular nuclei of frogs of one species to another species.

F. Val'ttser [transliterated] (USA) told of biochemical studies of sea urchin hybrids which were made by him jointly with P. Chen and A. Vitli [transliterated]. Each type of hybrid was characterized by peculiarities in the tempo of synthesis of protein, the amino acid composition of proteins, morphological character, and potential possibilities for development. The hybrids were also distinguished by the rate of synthesis of DNA after fertilization.

Zh. Emmerling (West Germany) devoted his paper to a study of the growth and photosynthesis in nuclear and anuclear fragments of acetabularia. He directed the attention of the participants in the symposium to the so-called morphogenetic substance on which the growth of a cell depends. He emphasized that there cannot be growth without synthesis of protein even though synthesis of proteins often takes place in the absence of growth. Experiments showed that the precursor of the morphogenetic substance of a photosynthesizing cell is synthesized even in the absence of light, but only in light can this substance display its activity in respect to growth of the cell. Emmerling considers that control by the nucleus over the synthesis of protein and the synthesis of the morphogenetic substance are different. It is probable that the morphogenetic substance cannot be synthesized in anuclear fragments but they are stored in them and stimulate the growth of anuclear cells.

K. Beta (USA) told of experiments on transplantation in acetabularia. Binuclear, trinuclear, or anuclear parts of the algae were joined together. A study was made of the length of the plants, the diameter and area of the Shapochka [literally little cap], the number of leaves, their dimensions, et cetera. Plants which contain two or

three nuclei compare with tetraploid and hexaploid [Sic]. Increasing the number of nuclei caused an increase in the size of the algae, the number of shapocha, the number of leaves and their size.

The paper by M. Alfert (USA) was deovted to quantitative cytochemical studies of DNA, basic proteins, the total quantity of proteins, and the volume of nuclei in the cells of animal tissues. The quantity of DNA and protein in the nuclei of cells of proliferating tissues increased per unit of nuclear volume. When there was physiological activity not connected with multiplication, the DNA remained unchanged while the protein content usually dropped.

Zh. Danielli (Great Britain) dwelt upon the problems of control over cell development by the nucleus and the cytoplasm, starting with experiments on transplanting nuclei in amoebae. He considers that nuclear control determines the nature of the cell and cytoplasmatic control its organization. The mechanism of interaction of the nucleus and the cytoplasm was tentatively connected with migrations of RNA from the nucleus to the cytoplasm and vice versa.

A very intersting paper was presented by M. Errer [transliterated] (Belgium) on nuclear-cytoplasmatic relationships in irradiated cells. Experiments were conducted on isolated nuclei of the thyroid gland and liver, and cells, and nuclear and anuclear fragments of amoebae and acetabularia. Irradiation of isolated nuclei decreased the inclusion of tagged amino acids into the proteins of the nuclei. It is interesting that adding irradiated cytoplasmatic structures to isolated nuclei also decreased the synthesis of proteins in the nuclei. Anuclear fragments of acetabularia and amoebae were more sensitive to radiation than nuclear fragments.

Discussion of the problem of nuclear-cytoplasmatic relationships, one of the most important problems of modern cytology, showed that at present any cytological study turns out to be connected to some extent with study of the biochemical processes and the chemical organization of the cells. The characteristic tendency in the development of cellular biology now is not only a rapprochement but an almost complete merging of cytology and cellular biology.

The work of the symposium was well organized. Papers were subjected to comprehensive discussion immediately after they were presented. Participants in the symposium were able to become familiar in detail with the work of the laboratories of Professor Brachet and Professor Shantren [transliterated] in the University of Brussels. Materials of the symposium will be published in one of the issues of the journal <u>Experimental Cell Research</u> of 1959.

CONFERENCE ON ADAPTATIONAL REACTIONS AND METHODS OF INCREASING RESISTANCE OF THE ORGANISM TO UNFAVORABLE INFLUENCES

I. P. Suzdal'skaya
V. P. Troshina

The Scientific Conference on the Problem of Adaptational Reactions and Methods for Increasing the Resistance of an Organism to Unfavorable Influences was held from 1 through 4 December 1958 in Leningrad at the Military Medical Academy imeni S. M. Kirov. About 500 scientific workers: physiologists, pharmacologists, cytologists, and microbiologists from different cities of the Soviet Union took part in the conference. Fifty two papers were presented. Two principal types of problems were examined at the conference--adaptational reactions of the whole organism and adaptational reactions of cells and tissues. The largest number of papers was devoted to adaptational reactions of the whole organism. The leading papers on this subject were given by N. V. Lazarev and I. R. Petrov.

N. V. Lazarev's paper expressed an interesting thought on the similarity of the phenomena which take place, on one hand, with "hardening" and "getting accustomed" to unfavorable environmental factors, and on the other hand, the effect on the organism of pharmacological preparations which increase its resistance. N. V. Lazarev believes that these preparations tend to stimulate the very same mechanisms which are also activized under the influence of unfavorable environmental factors on the organism. I. R. Petrov emphasized the nonspecific character of the increase in resistance of the organism to different stimuli. Thus, an organism adapted to low temperatures possesses increased resistance not only to cold, but also to oxygen insufficiency. General adaptational reactions were also observed in various pathological processes (excitation of the central nervous system accompanied by intensification of various vegetative functions and exchange of substances or inhibition accompanied by reduction of exchange).

Information from a large number of authors on the influence of different substances on increasing the resistance of organisms to unfavorable influences of the most varied nature served to illustrate these papers. The greatest attention was devoted to the effect of dibazol, vitamin B_{12}, and ginseng preparations. Administration of dibazol or vitamin B_{12} to animals weakened the inhibitory action on the central nervous system of large doses of adrenalin (G. P. Pospekhova), chlorpromazine (P. Dashnyam); increased the resistance of experimental animals to high temperature and dampness of the air (F. T. Agarkov, and to hypoxia (M. A. Rozin); increased the survival percentage of animals subjected to operative trauma (Ye. G. Vodokhlebova), and to electrical trauma and sodium cyanide poisoning (S. M. Vishnyakov). Prophylactic administration of dibazol or ginseng infusions to animals increased their resistance to rapid lowering of atmospheric pressure (V. G. Ovcharov),

to the action of carbon monoxide and potassium cyanide (Hei. Gen-y), and to the action of x-rays; the resistance of the animal organism was increased to the effect of various industrial poisons (Chang Ying-shany and the duration of alcoholic narcosis was shortened (Chan-fan). The antinarcotic effect of dibazol was also observed when the partial pressure of nitrogen was increased (I. S. Karev).

Papers which stated that the preliminary administration of dibazol could increase the resistance of animals to the action of viruses (S. A. Burov and P. I. Remezov) and pneumococci (I. M. Ivanushkin) were of particular interest. The most effective dose turned out to be 10 milligrams per kilogram of weight of the animal. An analogous phenomenon was also observed in man: the use of dibazol increased the resistance of the organism to inflammation of staphiloccocal etiology, to [a] virus group, to seasonal catarrh of the upper respiratory passages and made possible the production in the organism of antibodies against typhoid fever, A and B paratyphoid, and Sonne's dysentery (A. M. Kapitanenko). The paper by O. V. Bukharina and V. I. Dergacheva showed that the administration of vitamin B_{12} to dogs also led to intensified production of antibodies and increased the phagocytal activity of leucocytes. It was noted that dibazol prevented the development of fatigue in people (R. A. Okunev) and weakened late toxicoses in pregnancy (Ye. B. Derankova). Dibazol and Sintazol [synthazol] also restored sensitivity to light (N. T. Fedorov and L. N. Aleksandrov).

Increased resistance of the organism was also observed against the action of a number of other substances, such as: proserine and phenamine (M. A. Rozin), sodium lactate (N. T. Fedorov and Ye. A. Zakhariya), sulfur-containing substances--Etilizotiuroniya [ethyl isothiouronia], mercamine (Ye. A. Mukhin), metacil, pentoxyl, and cytosine (I. O. Grekh), fatty acids and phosphatides (B. I. Kadykov), certain preparations of plants of the composite flower family (B. E. Kolla). Increased nonspecific resistance was also discovered against unfavorable environmental factors--trauma, burns (V. K. Kulagin, V. B. Lemus), habituation to narcotics (Ye. I. Lyublina and I. V. Olyunin), ultraviolet irradiation (V. M. Matoshin), hyperoxia (A. G. Panov and P. I. Remezov) and hypooxia (F. T. Agarkov, T. A. Vasil'yev). N. V. Lauer and A. Z. Kolchinskaya showed the dependence of the resistance of the organism to oxygen insufficiency upon age. In some papers (M. A. Rozin, Z. I. Barbashova, K. A. Meshcherskaya, M. T. Golitsinskaya) an attempt was made to discover the mechanism of the nonspecific effect of medicinal substances and actions upon the organism.

A portion of the papers was devoted to the problem of the relationship between the specific and the nonspecific in reactions of the entire organism. The formation and development of specific reactions of the organism in ontogenesis was discussed in a paper by I. A. Arshavskiy. B. M. Fedorov acquainted the audience with data on the role of the nervous system in forming the reaction of the organism in response to the administration of toxic substances and the specificity of the

changes observed in the action of the heart at such times. Data on the nonspecific character of the effect of the most widely different pharmacological substances and actions on animal and human organisms were supplemented by a number of items which appeared in discussions. T. M. Sologova told of the ability of dibazol to increase the resistance of the organism against typhus--when it was administered the time of death of animals was moved back and the percentage of survival was increased. L. L. Vasil'yev told of mitigating the severity of the course of anaphylactic shock and infectious diseases in animals caused by diptheria toxin and intestinal bacteria by means of negatively ionized oxygen. L. L. Vasil'yev directed special attention of the audience to the ability of ionized oxygen to increase the resistance of tissues separated from the organism (based on data from the work of Kruger and Smith, United States).

Yu. M. Olenov reported on changes in the resistance of insects against the injurious action of insecticides which have been used against a series of successive generations. It was shown that lines which were resistant to DDT were sensitive to phosphoro-organic insecticides. Increase in resistance to DDT is the result of selection. The method of individual selection permitted increasing the resistance more than 100 times in several generations. The high resistance of the selected lines was caused by rapid mobilization of defense mechanisms.

The problem of adaptation of cells and tissues (we include here work done on protozoa and bacteria) aroused lively interest in the audience and merit detailed elucidation in the cytological journal. The first in this series was a paper by L. L. Vasil'yev on the subject "Functional Stability and Adaptation in the Light of the Study of Parabiosis." In it were presented data which proved that adaptation is a property not only of the whole organism, but also of surviving tissues and cells separated from the organism. The speaker illustrated his position by means of numerous facts obtained with a nerve-muscle preparation from a frog in the process of development of the threshold cathode parabiosis of the nerve. The adaptational capacity of the nerve first increased with the development of parabiosis, then decreased and went to zero. Here the period of increase in functional stability and adaptational capacity corresponded to the prodromic phase of the parabiotic process--the more clear-cut it is, the greater the adaptational capacity and the slower the parabiotic process. Changes in the adaptational capacity of nerve-muscle formations caused by specific agents (allergies)" are also subordinated to the general patterns of the parabiotic process.

B. P. Ushakov's paper was devoted to the problem of adaptation of cells of the animal organism. In this paper were presented materials which indicated that adaptation of cells, defined as their defensive reaction, may be realized in two ways: 1) an increase in the stability of the proetin structure of the cell; 2) inclusion of the exchange processes which regulate the reactivity of proteins within the cells. A comparative cytophysiological analysis of the adaptational process showed that in the process of evolution adaptational changes in the protein

structure of cells were produced in accordance with changes in the environment of the animals and the adaptational intracellular regulatory mechanisms were intensified. In concluding B. P. Ushakov emphasized that adaptation of the organism was by no means always linked with radical reconstruction of the organism, including reconstruction of all its cells, but in many cases the adaptational process was realized by conserving the basic mass of cells and proteins of the organism.

The paper by V. Ya. Aleksandrov contained a discussion of autoregulatory phenomena which appeared when cells were exposed to the injurious action of high temperature. On the basis of a large amount of experimental material, the speaker came to the conclusion that cells are capable of increasing their stability in response to the injurious action of different agents. Thus, the action of excessively high temperatures (30-44 degrees) increased the heat stability of plant cells, and at the same time increased the resistance to ethyl alcohol, to acetic acid, and to high hydrostatic pressure, but did not increase resistance to ammonia. Consequently, a wide range of nonspecific increase in the resistance of cells takes place along with thermal hardening. Still another type of increase in stability exists in protozoa--"adjustment." It occurs through the action of temperatures which are not harmful and excessive, but which are within the bounds of the zone of tolerance. Then there is an "adjustment" in which the heat stability of the protozoa depends directly upon the temperature of their upbringing (refer to works by Yu. P. Polyanskiy). The capacity for "adjustment" to temperature is lacking in the cells of animal and plant tissues.

Papers by M. B. Kiro, N. A. Vinogradova, and N. B. Il'inskaya were devoted to individual problems of tissue adaptation. N. B. Il'inskaya showed that when striated muscle tissue from frogs was exposed to the action of weak urea solutions, adaptational processes were manifested in disappearance of the initial contraction. Muscles left in a solution of urea are weakened, and the excitability that had disappeared is restored. These adaptational phenomena do not occur when solutions with more than 9 per cent of urea are used. M. B. Kiro's paper stated that when weak concentrations of the most varied substances (salts, narcotis, sugars, methylene blue, penicillin) acted on isolated tissues (muscle tissue, ciliated epithelium) he discovered an increase in resistance, judged on the basis of the time of survival in solutions of the agents under study. Neither bactericidal action nor tonic nature of the solutions can explain the results obtained. The speaker believes that the increase in resistance is a manifestation of the first phase of paranecrosis characterized by a decrease in sorptional capacity of the tissues.

It also turned out to be possible to observe a phase of increased resistance, revealed by another method, in isolated frog muscles kept in Ringer's solution at room temperature. N. A. Vinogradova spoke of this in her paper. Immediately after the muscle was isolated from the organism, long before the beginning of contracture, the functional and substantial properties of the muscle began to change and this change had a phasal character. An increase in potential difference, an increase in

excitability, and a decrease in sorptional capacity were observed in the phase of increased resistence of the muscles to the action of high temperature.

L. N. Seravin reported on a similar phasal (waveform) change in many functions of infusoria in the process of becoming accustomed to chemical agents. Thus, under the action of chloride salts of calcium and sodium the resistance of protozoa first decreased, then increased strongly, after which it again began to decrease. The rate of phagocytosis of paramecia also changed in a three-phase pattern. Changes in different functions in the process of becoming accustomed to chemical agents proceeded at different rates and not in parallel. In the process of becoming accustomed to the active agent, the upper threshold of the rate of survival of paramecia increased, that is, they began to survive in concentrations of the agent which were lethal to the control. The speaker regarded habituation as one of the basic forms of direct adaptation of organisms and cells to new environments leading to an increase in the stability of organisms and cells and ensuring complete or at least partial restoration of their basic functions initially destroyed by changes in the environment.

L. K. Lozina-Lozinskiy reported on the adaptation of infusoria to the action of radiant energy (ultraviolet, ionizing radiation) and to the photodynamic action of light. The speaker directed attention to the fact that adaptation proceeds through an increase in the stability of different functions of the cell which are chiefly under the control of either the nuclear apparatus or of the cytoplasm. Starting with the different sensitivity and reactivity of cellular components, Lozina-Lozinskiy considers it essential to separate nuclear and cytoplasmatic resistance and adaptation. The speaker reported on experiments which he conducted jointly with S. N. Aleksandrov in which a paramecium population taken from a radioactive source turned out to be more resistant to a large doses of x-rays than the control. In the author's opinion, this resistance depends rather on the repair capacities of the cytoplasm rather than of the nucleus. It was also shown in the paper that the adaptation of infusoria can be realized without changes in their resistance as a result of changes in the "behavior" of the infusoria. Thus, under definite intensities of "white" light and concentrations of photodynamic color, a negative photokinetic reaction occurred in paramecia, due to which they avoided the injurious effect of the color by leaving for a zone where the rays which caused photosensitizing oxidation did not penetrate.

The paper by L. G. Perets was devoted to the adaptational reactions of microorganisms. The speaker emphasized the importance of studying nonspecific resistance of microbes to external influences. When microbes acquire resistance to any sort of specific agent they can also change at the same time their reaction to other influences. Thus, for example, when pathogenic microbes acquire resistance to sulfanilamide preparations or to antibiotics, their most varied biological

properties change--the intensity of multiplication increase, and resistance to different physical, chemical, and biological factors increase to a marked degree. At the same time, virulence most frequently is reduced. The reduction seen in recent years in the severity of certain diseases--scarlatina, dysentery, pneumonia, and others is essentially connected with this change.

The papers on problems of adaptation of cells, tissues, and the most simple organisms brought about an extensive exchange of opinions among the participants in the conference. In his summary speech, N. V. Lazarev concentrated attention on the nonspecificity of the processes of adaptation. He noted that biologists, in particular, that cyto-physiologists rather than pharmacologists were first to see in isolated tissues that which was repeated and in higher organisms, nonspecificity of adaptation. Up to the present time pharmacology had been occupied with searching for specific remedies and did not undertake the task of increasing the stability of organisms by nonspecific means. Now it is necessary to test another way in healing--the use of nonspecific means. This gives rise to the problem of searching for medicines of a wide range of action. The papers read at the conference showed that oxygen insufficiency might perhaps turn out to be such a medicine. Establishing a connection between increased resistance and length of life is a task for future studies. The statement by Sel'ye [transliterated] that the greater the adaptation, the greater the expenditure of energy, and the shorter the life is not justified, judging by our data. L. L. Vasil'yev emphasized that it is well known in cellular physiology that the reactions of adaptation and sensitization are nonspecific. However, this fact, as the conference showed, is something new when applied to the whole organism.

I. A. Arshavskiy noted the general physiological interest and value of the papers on adaptation of cells and tissues. He believes that the concept of homeostasis may be applicable to cellular forms. In the opinion of I. A. Arshavskiy, the conference again showed that there is an urgent need for unification of the terminology used.

V. Ya. Aleksandrov remarked that contradictory terminology was connected with the different viewpoints of scientists on the essence of the matter and suggested that the terms used and the viewpoints of different authors on problems of adaptation be compared in a special paper at the next conference. V. Ya. Aleksandrov, like other previous speakers, expressed his satisfaction in regard to the fact that pharmacologists and cytologists had discovered common interests due to elements of similarity in reactions of cells and multicelled organisms, and stated his certainty that exchange of experience by pharmacologists and cytologists would enrich both branches of science. V. Ya. Aleksandrov directed attention to the fact that an organism could react expediently to an injurious influence which neither it nor its precursors had ever encountered. He believes that to explain this, one must start with ideas of the presence of nonspecific patterns in the action of heterogeneous injurious factors.

In his speech N. V. Golikov noted the well-planned program of the conference, as a result of which the problems of reactivity and resistence were elucidated on both the cellular level and on the level of systemic reactions of the entire organism. According to N. V. Golikov, L. L. Vasil'yev's paper should have discussed the facts of increasing resistence not only with increased lability, but also with reduced lability and polarization. N. B. Shlyakhter's speech gave an example of nonspecific increase of resistance of isolated muscles under the influence of temperature. V. P. Paribok told of analogous phenomena which had occurred in isolated intestinal tissue under the action of alcohol. He suggested a wider use of the duration of life of isolated tissues, organs, and whole organisms as a criterion of stability in respect to the action of different agents.

Z. I. Barbashova noted that the data presented at the conference indicated the general biological character of the reaction of nonspecific increase in resistance. She pointed out that increases in resistance as a result of the action of various agents could be different. Therefore, it was extremely important to take into account the amount of the stabilizing or sensitizing agent. According to Z. I. Barbashova, a search for agents which would increase the resistance of the tissues themselves is now one of the fundamental tasks of research in the field of adaptational processes. A. G. Subbota directed the attention of the conference to the presence of the very same adaptational mechanisms for ordinary and for unusual stimuli. Thus, many papers emphasized an increase in the resistance of organisms to unfavorable influences with oxygen starvation. G. S. Strelin noted the urgency of the problem of adaptation to radiation and reported on cases of adaptation to this agent when its action was continuous. He believes that adaptation to radiation occurs as a result of increased production of blood.

A. A. Sinitskiy expressed skepticism in regard to combatting infectious diseases with nonspecific remedies. He believes that a great deal more data are required to justify the usefulness of nonspecific remedies like dibazol for prophylaxis. A. A. Sinitskiy's speech gave rise to lively discussion, in which L. G. Perets, I. M. Ivanushkin, and other participated. They upheld the idea of the expediency of increasing the general stability of the organism in respect to the action of nonspecific stimuli during specific infections. In the concluding speech N. V. Lazarev pointed out the urgent need for solving the problem of nonspecific adaptation of animals to different unfavorable factors. It has turned out that there is a full reflection in the macroorganism of that which co-workers of the school of N. Ye. Vvedenskiy and D. N. Nasonov have observed in cellular and tissue objects. Nonspecific reactions to external influences exist on both the cellular level and on the level of the organism. The significance of this conference is that for the first time pharmacologists, physiologists, cytologists who have been interested in the development of these ideas have become acquainted with each other and have seen how much their scientific interests have in common. There exists an adaptational syndrome which

is caused by special agents and which aids the organism to achieve a high level of resistance. The fact that we control the syndrome of increase of resistance merits special attention. The clarification of the mechanisms for increasing resistance is the basic and essential task of the present time.

In concluding our survey, it is necessary to add that the problems of increasing resistance are exceedingly urgent and the exchange of opinions which took place at the conference will undoubtedly serve as a point of departure for new studies which will be important for theory and for practice.

FOR THE INFORMATION OF WRITERS

The editorial staff of the journal Tsitologiya requests that writers be guided by the rules set forth below when sending articles to the journal. Articles which have been sent in without observance of the rules set forth below will not be accepted.

1. Articles on the cytology of animals and plants, both one-celled and multicelled, are published in this journal. The journal elucidates all the basic fields of the science of the cell: problems of morphology, the physiology and biochemistry of the cell, cytogenetics, radiation cytology, the cytology of one-celled organisms, cytoecology, also the application of cytological research in fields of medicine and agriculture. In the journal are printed previously unpublished original papers, survey articles, articles of a discussional character, criticism, bibliographies, news items, articles on the history of cytology, and reports on new methods of cytological research. Preliminary reports are not accepted.

2. Articles should be written concisely, clearly, and should be painstakingly edited. Repetition of the same data in the text, tables, and figures is not permitted. The size of manuscripts should not, as a rule, exceed 16 typed pages, with a minimum number of illustrations.

3. Articles must be double-spaced on white writing paper, on one side of the sheet, with left margins of not less than 4 centimeters, and are to be presented to the editorial staff in two copies (one must be the original copy). Places where drawings and tables should be placed are to be marked in the margin. All pages of the manuscript should be numbered. Tables, bibliography, and figure captions should be printed on separate sheets.

4. Formulas should be written in ink or India ink, precisely, without admixtures of occasional typed letters, in library handwriting. Indices and exponents must be indicated with particular accuracy--hachures, commas, and ones must be clearly distinguishable from each other. It is necessary to make a clear distinction between capital and lower case letters; in cases where they are identical, in outline, it is necessary to underline capital letters with two lines (for example, $\underline{\underline{C}}$),

and to place two lines above non-capitals ($\bar{\bar{c}}$). It is necessary to distinguish between 1 and the letter l, O, o, and 0 (zero); I and J, et cetera.

5. The manuscript should be signed by the author (by all co-authors) and be accompanied by a permit from the institution where the work was accomplished. After the name of the article, the initials and surnames of the authors, the name of the laboratory, institution, and city where the work was accomplished are to be listed. At the end of the article it is necessary to give the precise address, position, surname, first name and patronymic, also the telephone number of the author.

6. Original articles must end with brief conclusions or a resume.

7. The surnames of foreign authors are to be given in the text in Russian letters; in references to works, in the original letters with the year of publication of the work indicated, for example: Overton, 1895 observed... "References to works by several writers are to be given in chronological order of publication of works, for example: Many authors (Zavarzin, 1927; Ries, 1938; Nasonov, 1939; Roskin and Brodskiy, 1953, and others) described ...".

8. Footnotes should have successive numbering for the entire article. Cited literature should be listed at the end of the article. This bibliography is to include only works mentioned in the text of the article. All works mentioned in the text of the article should be listed in the bibliography. The list is to contain in alphabetical order first the literature in the Russian language, then that in foreign languages. A work by a Russian author published in a foreign language is to be included in the list of works in the Russian language, but the surname and initials of the author are to be given in Russian in parentheses preceding the foreign letters.

9. The bibliography is to be composed in the following form: a) for books--after the surname and initials of the author, give the year of the edition, the full title of the book and place of publication, for example: Zavarzin, A. A. 1945. Ocherki evolyutsionnoy gistologii krovi i soedinitel'noy tkani. Essays on the Evolutionary Histology of the Blood and Connective Tissue, First Edition, Moscow; b) for articles--the surname and initials of the author are to be followed by the year, then the name of the article, the name of the journal or collection of articles, the volume, the number or issue, and the pages, for example: Fenn, W. and Haege, L. 1942. "The Penetration of Magnesium into Frog Muscle," J. Cell. Comp. Physiol. 19, 1 : 37-46.

10. Drawings should be made clearly. They are to be attached to the article in a separate envelope. Photographs are to be sent in two copies on glossy paper. Microphotographs should have rectangular outlines. Give the scale of microscopic illustrations and indicate the power of objective and eyepiece in captions of drawings. All symbols are to be given on one copy of the photograph, the second copy is to remain clear. As far as possible, inscriptions on figures [drawings] are to be replaced by numerals or letters, the meaning of which is

to be listed in the caption. On the back of the drawing indicate in pencil the surname of the author, the title of the article, the number and the desired reduction of the figure.

11. Tables and figures should be assigned ordered numbers to which reference can be made in the text of the article. All graphs in tables and the tables themselves should be supplied with headings. Abbreviated words are not permitted in the tables.

12. Use must be made in the text and in the bibliography of accepted abbreviations of individual words, units of measurement, titles of journals, et cetera. A list of these abbreviations is given in the handbook Podgotovka rukopisi k naboru [Preparation of Manuscripts for Printing] (Moscow, Publishing House of the AN, USSR, 1958).

13. The editorial staff reserves the right to condense articles and to insert editorial corrections after agreeing with the authors on the changes.

14. It is possible to send a duplicate copy of the manuscript prepared for printing for checking and corrections of errors instead of proofs; no changes or additions will be permitted. The duplicate of the manuscript with the signature of the author and the date of signature should be sent back to the editorial office within two days after it has been received.

15. When a manuscript is returned to the author for revision, the date of its receipt is retained for two months.

16. In case the article is rejected, one copy of the manuscript may be returned to the author.

17. The address of the editorial office is: Leningrad, F-121, pr. Maklina 32, Editorial Office of the Journal Tsitologiya. Telephone D-1-51-67.

CORRECTION

Pages 134 and 135 of No 1 of the journal contain errors. The first meeting of the session of cytologists in the Sixth All-Union Congress of Anatomists, Histologists, and Embryologists in Kiev was held under the chairmanship of P. V. Makarov, not G. I. Roskin, who held the chairmanship of the next meeting of the session.

ACADEMY OF SCIENCES OF THE USSR DEPARTMENT OF BIOLOGICAL SCIENCES

COMPETITION FOR NAMED PRIZES OF THE AN, USSR IN 1959

The Department of Biological Sciences of the AN, USSR reports that contests will be conducted in 1959 for the named prizes:

1. The V. R. Vil'yams Prize, in the amount of 10,000 rubles, will be awarded to Soviet scientists for the best scientific works in the field of soil-agronomic sciences.

2. The I. M. Sechenov Prize, in the amount of 20,000 rubles, will be awarded to Soviet scientists for outstanding experimental and theoretical research in the field of generaly physiology.

Only Soviet scientists, both individuals and groups of authors, can participate in the contests.

Works entered in competition for the named prizes may be submitted by scientific societies, scientific research institution, institutions of higher learning, honorary members, active members, and corresponding members of the Academy of Sciences, USSR.

Only published works will be submitted in competition.

Works in competition for the named prizes will be submitted to the Department of Biological Sciences of the Academy of Sciences, USSR (Moscow, Leninskiy Prospekt, 14) in any language in three copies with the superscription "In competition for the ...... Prize."

The following must be attached to works:

a) report on the scientific work, with a volume of up to 0.25 author's sheets;

b) material on judgment of submitted works for the scientific community;

c) brief biographical reports on authors and a list of their scientific works and inventions.

The deadline for submitting works in competition for the named prizes--up to 15 September 1959.

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